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The epidemiology of severe *Streptococcus pyogenes* disease in Europe

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Department of Bacterial and Inflammatory Diseases,
National Public Health Institute, Helsinki, Finland

Haartman Institute, University of Helsinki, Finland

Health Protection Agency Centre for Infections, London, UK

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Theresa Lamagni

ACADEMIC DISSERTATION

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ABSTRACT

Diseases caused by the Lancefield group A streptococcus, *Streptococcus pyogenes*, are amongst the most challenging to clinicians and public health specialists alike. Although severe infections caused by *S. pyogenes* are relatively uncommon, affecting around 3 per 100,000 of the population *per annum* in developed countries, the case fatality is high relative to many other infections. Despite a long scientific tradition of studying their occurrence and characteristics, many aspects of their epidemiology remain poorly understood, and potential control measures undefined.

Epidemiological studies can play an important role in identifying host, pathogen and environmental factors associated with risk of disease, manifestation of particular syndromes or poor survival. This can be of value in targeting prevention activities, as well directing further basic research, potentially paving the way for the identification of novel therapeutic targets. The formation of a European network, Strep-EURO, provided an opportunity to explore epidemiological patterns across Europe.

Funded by the Fifth Framework Programme of the European Commission's Directorate-General for Research (QLK2.CT.2002.01398), the Strep-EURO network was launched in September 2002. Twelve participants across eleven countries took part, led by the University of Lund in Sweden. Cases were defined as patients with *S. pyogenes* isolated from a normally sterile site, or non-sterile site in combination with clinical signs of streptococcal toxic shock syndrome (STSS). All participating countries undertook prospective enhanced surveillance between 1st January 2003 and 31st December 2004 to identify cases diagnosed during this period.

A standardised surveillance dataset was defined, comprising demographic, clinical and risk factor information collected through a questionnaire. Isolates were collected by the

national reference laboratories and characterised according to their M protein using conventional serological and *emm* gene typing.

Descriptive statistics and multivariable analyses were undertaken to compare characteristics of cases between countries and identify factors associated with increased risk of death or development of STSS. Crude and age-adjusted rates of infection were calculated for each country where a catchment population could be defined.

The project succeeded in establishing the first European surveillance network for severe *S. pyogenes* infections, with 5522 cases identified over the two years. Analysis of data gathered in the eleven countries yielded important new information on the epidemiology of severe *S. pyogenes* infections in Europe during the 2000s. Comprehensive epidemiological data on these infections were obtained for the first time from France, Greece and Romania. Incidence estimates identified a general north-south gradient, from high to low. Remarkably similar age-standardised rates were observed among the three Nordic participants, between 2.2 and 2.3 per 100,000 population. Rates in the UK were higher still, 2.9/100,000, elevated by an upsurge in drug injectors. Rates from these northern countries were reasonably close to those observed in the USA and Australia during this period. In contrast, rates of reports in the more central and southern countries (Czech Republic, Romania, Cyprus and Italy) were substantially lower, 0.3 to 1.5 per 100,000 population, a likely reflection of poorer uptake of microbiological diagnostic methods within these countries.

Analysis of project data brought some new insights into risk factors for severe *S. pyogenes* infection, especially the importance of injecting drug users in the UK, with infections in this group fundamentally reshaping the epidemiology of these infections during this period. Several novel findings arose through this work, including the high degree of congruence in seasonal patterns between countries and the seasonal changes in case fatality rates. Elderly patients, those with compromised immune systems, those who developed STSS and those infected with an *emm*/M78, *emm*/M5, *emm*/M3 or *emm*/M1 were found to be most likely to die as a result of their infection, whereas those diagnosed with cellulitis, septic arthritis, puerperal sepsis or with non-

focal infection were associated with low risk of death, as were infections occurring during October. Analysis of augmented data from the UK found use of NSAIDs to be significantly associated with development of STSS, adding further fuel to the debate surrounding the role of NSAIDs in the development of severe disease.

As a largely community-acquired infection, occurring sporadically and diffusely throughout the population, opportunities for control of severe infections caused by *S. pyogenes* remain limited, primarily involving contact chemoprophylaxis where clusters arise. Analysis of UK Strep-EURO data were used to quantify the risk to household contacts of cases, forming the basis of national guidance on the management of infection. Vaccines currently under development could offer a more effective control programme in future.

Surveillance of invasive infections caused by *S. pyogenes* is of considerable public health importance as a means of identifying long and short-term trends in incidence, allowing the need for, or impact of, public health measures to be evaluated. As a dynamic pathogen co-existing among a dynamic population, new opportunities for exploitation of its human host are likely to arise periodically, and as such continued monitoring remains essential.

Keywords: *Streptococcus pyogenes*; Fatal Outcome; Shock, septic; Seasons; Bacteraemia; Streptococcal vaccines; Communicable disease control; Population Surveillance; Epidemiology; Europe, UK.

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TIIVISTELMÄ

Lancefield-ryhmän A streptokokin eli *Streptococcus pyogenes*-bakteerin aiheuttamat taudit ovat erittäin haasteellisia kliinikoille sekä kansanterveyden asiantuntijoille. Vaikka *S. pyogenesin* aiheuttamia vakavia infektioita esiintyy kehittyneissä maissa väestöpohjaisesti vain kolmella 100 000:sta vuosittain, on tapauskuolleisuus suuri verrattuna moniin muihin infektiotauteihin. Siitä huolimatta, että näiden infektioiden ominaisuuksia ja esiintymistä koskevalla tutkimuksella on pitkät perinteet, on niiden epidemiologia vielä useilta osin huonosti tunnettua ja mahdolliset torjuntakeinot määrittelemättä.

Epidemiologisilla tutkimuksilla voi olla merkittävä rooli tautiriskiin, tiettyihin oireisiin tai huonoon selviytymiseen liittyvien isäntään, taudinaiheuttajaan ja ympäristötekijöihin liittyvien tekijöiden tunnistamisessa. Tämä voi osoittautua hyödylliseksi ehkäisytoimenpiteitä ja jatkotutkimuksia suunnitellessa, sekä toimia uraauurtavasti uusien hoidon kohteiden tunnistamisessa. Eurooppalaisen Strep-EURO- verkoston perustaminen mahdollisti epidemiologisten tekijöiden tutkimuksen ympäri Eurooppaa.

Euroopan komission tutkimusdirektoraatin (QLK2.CT.2002.01398) viidennen puiteohjelman rahoittama Strep-EURO -verkosto perustettiin syyskuussa 2002. Hankkeeseen osallistui kaksitoista osanottajaa yhdestätoista maasta ruotsalaisen Lundin yliopiston johdolla. Tapauksiksi määriteltiin potilaat, joilta oli viljelty *S. pyogenes* joko normaalisti steriilistä tai epästeriilistä kohteesta yhdistettynä toksisen shokin taudinkuvaan (STSS). Prospektiivisen tehoseurantatutkimuksen avulla etsittiin kaikista osallistujamaista 1. tammikuuta 2003 ja 31. joulukuuta 2004 välisenä aikana diagnostisoituja potilastapauksia.

Luotiin standardisoidu seurantatietokanta, joka sisälsi kyselylomakkeen avulla kerättyjä demografisia, kliinisiä ja riskitekijätietoja. Kansalliset asiantuntijalaboratoriot keräsivät bakteerilöydökset, ja tutkivat niiden M proteiineja perinteistä serologista ja *emm* -geenityypitystä hyödyntämällä.

Deskriktiivinen tilasto- ja monimuuttuja-analyysi suoritettiin eri maiden välillä tautitapauksiin liittyvien ominaisuuksien sekä suurentuneeseen kuolemanriskiin tai STSS:n kehittymiseen liittyvien riskitekijöiden tunnistamiseksi. Infektioiden esiintymisluvut laskettiin sekä suoraan että ikäryhmiin mukautettuna kullekin maalle, jonka kohdeväestö oli määriteltävissä.

Tunnistamalla 5522 tapausta kahden vuoden aikana, hanke onnistui luomaan ensimmäisen eurooppalaisen vakavien *S. pyogenes* infektioiden seurantaverkoston. Yhdessätoista maasta kerättyä materiaalia analysoimalla saatiin uutta, tärkeää tietoa vakavien *S.pyogenes* infektioiden epidemiologiasta Euroopassa 2000-luvulla. Kattavaa epidemiologista tietoa näistä infektioista oli saatavilla ensimmäistä kertaa Ranskasta, Kreikasta ja Romaniasta. Tapausten esiintymistiheys viittasi korkeasta alhaiseen kulkevaan pohjois-etelä gradienttiin. Kolmen pohjoismaalaisen osallistujan välillä oli nähtävissä huomattavan yhdenmukaiset ikävakioidut arvot, noin 2,2 -2,3 tapausta 100 000 asukasta kohti. Ruiskuhuumeita käyttävien äkillisesti nousseesta lukumäärästä johtuen olivat kyseiset luvut Iso-Britaniassa vielä korkeampia, noin 2,9/100 000. Näistä Pohjois-Euroopan maista saadut luvut vastasivat suunnilleen USA:sta ja Australiasta samalla aikavälillä saatuja arvoja. Euroopan keski- ja eteläosan maissa (Tsekin tasavalta, Romania, Kypros ja Italia) havaittiin sen sijaan huomattavasti vähemmän tapauksia ja kyseiset luvut vaihtelivat 0,3:n ja 1,5:n tapauksen välillä 100 000 asukasta kohti. Tämän voidaan mitä todennäköisimmin katsoa heijastuvan mikrobiologisten diagnostisten menetelmien vähemmästä käytöstä kyseisissä maissa.

Projektitulosten analysointi toi joitakin uusia näkökulmia vakavien *S. pyogenes* tautien riskitekijöistä. Etenkin Iso-Britanniassa ruiskuhuumeiden käyttö ja näiden potilaiden infektiot muokkasivat merkittävästi tautiepidemiologiaa tänä ajanjaksona. Tutkimus tuotti paljon uusia tuloksia, kuten vuodenaikavaihtelun samankaltaisuuden kaikissa maissa, ja

vuodenajan vaikutuksen tapauskuolleisuuteen. Iäkkäät ja puolustusrajoitteiset potilaat sekä henkilöt, joille oli kehittynyt STSS tai joilla oli *emm*/M78, *emm*/M5, *emm*/M3 tai *emm*/M1 tauti, kuolivat todennäköisimmin infektion seurauksena, kun taas selluliittia, septistä artriittia, puerperaalista sepsistä tai yleisinfektiota (ei elinfokusta) sairastavien potilaiden keskuudessa kuolemanriski oli alhainen. Kuolleisuus oli alhaisempaa myös, jos infektio esiintyi lokakuussa. Iso-Britannian laajennetun tietokeräysmateriaalin analyysissa NSAID lääkkeiden käyttö assosioitui tilastollisesti merkitsevästi STSS:n kehittymiseen. Tämä löydös saattaa osaltaan kiihdyttää keskustelua, jota käydään NSAID lääkkeiden roolista vakavan taudin kehittymiseen.

Koska vakavat *S.pyogenes* infektiot ovat pitkälti avohoitoperäisiä, ja ajallisesti ja maantieteellisesti harvakseltaan esiintyviä tauteja, ovat torjuntakeinot vähäiset. Ne rajoittuvat lähinnä lähikontaktien lääkeprofylaksiaan, jos todetaan tautirypäitä. Iso-Britannian Strep-EURO tuloksia käytettiin arvioimaan samassa taloudessa asuvien henkilöiden tautiriskiä; tämä loi pohjaa kansallisen hoito-ohjeen luonnille. Kehitteillä olevat rokotteet voisivat tulevaisuudessa tarjota tehokkaamman torjuntaohjelman.

Vakavien *S. pyogenes* infektioiden seuranta on kansanterveydellisesti tärkeää nimenomaan pitkän ja lyhyen ajanjakson esiintyvyydessä tapahtuvien muutosten tunnistamiseksi, sekä kansanterveydellisten toimenpiteiden tarpeen ja vaikutusten arvioimiseksi. *S. pyogenes,* joka on muuntautumiskykyinen taudinaiheuttaja, osaa väestössä ja isännässä tapahtuvien muutosten myötä etsiä aika ajoin uusia taudinaiheuttamismuotoja. Tämän takia jatkuva seuranta on tärkeää.

Avainsanat: *Streptococcus pyogenes*, kuolema; shokki; septinen; kausi/vuodenaika; bakteremia; streptokokkirokotteet; tartuntatautien torjunta; väestön seuranta; epidemiologia; Eurooppa; Iso-Britannia.

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The dissertation is based on the following original publications, which shall be referred to throughout the text by the Roman numerals given below (I-IV). Some unpublished data are also presented. The copyright holders granted permission for the articles to be reprinted.

I. Lamagni TL, Neal S, Keshishian C, Alhaddad N, George R, Duckworth G, Vuopio-Varkila J, Efstratiou A. Severe *Streptococcus pyogenes* infections in the United Kingdom, 2003-04. *Emerg Infect Dis* 2008, 14(2):202-9.

II. Lamagni TL, Darenberg J, Luca-Harari B, Siljander T, Efstratiou A, Henriques-Normark B, Vuopio-Varkila J, Bouvet A, Creti R, Ekelund K, Koliou M, Stathi A, Strakova L, van der Linden M, Ungureanu V, Schalén C, Strep-EURO study group, Jasir A. The epidemiology of severe *Streptococcus pyogenes* disease in Europe. *J Clin Microbiol* 2008, 46(7):2359-67.

III. Lamagni TL, Neal S, Keshishian C, Hope V, George R, Duckworth G, Vuopio-Varkila J, Efstratiou A. Epidemic of severe *Streptococcus pyogenes* infections in UK injecting drug users, 2003-04. *Clin Microbiol Infect* (in press).

IV. Lamagni TL, Neal S, Keshishian C, Potz N, Powell D, Pebody R, Duckworth G, George R, Vuopio-Varkila J, Efstratiou A. Predictors of mortality following severe *Streptococcus pyogenes* infection (submitted).

ABBREVIATIONS

BSAC	British Society for Antimicrobial Chemotherapy
CA-SFM	Committee for Antibiotic Susceptibility Testing, French Society of Microbiology
CDC	Centers for Disease Control and Prevention (USA)
C.I.	confidence interval
CLSI	Clinical and Laboratory Standards Institute (USA)
df	degrees of freedom
ECDC	European Centre for Disease Prevention and Control
EARSS	European Antimicrobial Resistance Surveillance System
ELISA	enzyme-linked immunosorbent assay
GAS	group A streptococcus
HPA	Health Protection Agency (UK)
ICD	International Classification of Diseases
IDU	injecting drug user
IVIG	intravenous polyspecific immunoglobulin G
KTL	Kansanterveyslaitos (National Public Health Institute, Finland)
MIC	minimum inhibitory concentration
NSAID	non-steroidal anti-inflammatory drug
OF	opacity factor
OR	odds ratio
PCR	polymerase chain reaction
RR	rate ratio
S. pyogenes	*Streptococcus pyogenes*
spe	streptococcal pyrogenic exotoxin gene
SRGA	Swedish Reference Group for Antibiotics
STSS	streptococcal toxic shock syndrome
WHO	World Health Organisation

1. INTRODUCTION

Diseases caused by the Lancefield group A streptococcus, *Streptococcus pyogenes*, are among the most varied in terms of clinical spectra and severity, ranging from the ubiquitous pharyngitis to rarer life-threatening presentations such as necrotizing fasciitis. Although severe infections caused by *S. pyogenes* are relatively uncommon, affecting around 3 per 100,000 of the population *per annum* in developed countries[1;2], the case fatality is high relative to many other infections, around 7-23%[2-8]. The rapidity with which patients can deteriorate bestows further notoriety to this pathogen[9-11], inducing disquiet among frontline medical staff faced with a differential diagnosis, and fear amongst the public at large. Although attributable mortality is higher among the elderly and those with impaired immune systems, deaths among the young and previously healthy are not uncommon[11-13].

Invasive *S. pyogenes* infections have attracted increasing levels of attention since the late 1980s when reports from the USA, Canada, Norway, Sweden and Denmark warned of a possible re-emergence of severe clinical manifestations of *S. pyogenes*, and non-suppurative sequelae such as rheumatic fever[9;11;14-19]. Serotype M1, and to a lesser extent M3, were generally implicated in these rises[10;16;20;21].

During the early 1980s reports emerged from the then Czechoslovakia and the USA describing a hitherto unrecognized complication of *S. pyogenes* infection, termed the 'streptococcal toxic shock-like syndrome'[6;12;22;23]. A review of these reports by a CDC working group led to the establishment of a case definition for streptococcal toxic shock-like syndrome (STSS)[24]. The diverse spectrum of invasive diseases recognised as being caused by *Streptococcus pyogenes* included puerperal sepsis, necrotizing fasciitis, septic arthritis, pneumonia, STSS and non-focal bacteraemia.

One of the most defining events for severe *S. pyogenes* disease surveillance activity occurred in 1994 when a cluster of necrotizing fasciitis cases was detected in Gloucestershire, in the South West of England[25]. This event acted as an important catalyst for a host of activity within and outside the UK. Enhanced surveillance for severe *S. pyogenes* disease was immediately implemented in the UK[26], with two other

European countries following suit[27;28]. This response resulted in a small number of countries obtaining for the first time measures of disease-specific incidence, risk factors and outcome.

The impetus generated during the mid-1990s led to the establishment of an *ad hoc* WHO working group on *Streptococcus pyogenes,* comprised of representatives from streptococcal reference centres in Canada, Czech Republic, Italy, New Zealand, UK and USA. The main recommendation of the ensuing WHO consultations was to support member countries in initiating comprehensive public health programmes for the control of severe *S. pyogenes* infections. The key priorities that emerged from the pivotal 1998 consultation included the urgent need to develop a mechanism to strengthen microbiological capacity and provide sustained support to an international network of laboratories, the need to evaluate the tools available for surveillance, and the need to embed streptococcal infections within national public health priorities[29]. However, no definitive network across Europe was formed, and collaborations between European countries were undertaken, if at all, on a largely informal basis. A European network was not established until 2002[30].

Despite the importance of these infections and the long scientific tradition of studying their occurrence and characteristics, many aspects of their natural history remain poorly understood, and potential control measures often undefined. Epidemiological studies can identify a range of host, pathogen and environmental factors associated with particular disease manifestations or with poor survival, which can in turn help direct further research at a cellular level, potentially paving the way for identification of novel therapeutic or preventative targets. Collecting an array of patient and microbiological data on at a sufficiently large and representative number of patients can, however, present a logistical and financial challenge. The formation of a European network, Strep-EURO, provided an opportunity to rise to this challenge[31].

2. REVIEW OF THE LITERATURE

2.1. *Streptococcus pyogenes* as a human pathogen

2.1.1. Discovery of *Streptococcus pyogenes*

Like other members of the family Streptococcaceae, streptococci are Gram-positive facultative anaerobic organisms which occur in chains or in pairs[32]. The name *Streptococcus* was proposed by Theodor Billroth in 1874, who identified these organisms from patients with erysipelas and wound infections[33;34]. The name was coined in recognition of the characteristic chain formation of the genus, from the Greek *streptos* for chain or twisted, and *kokhos* meaning berry or seed, referring to the globular-shaped particles[35]. The individual streptococcal species then became named after the diseases they caused or sites of infection[35], with *Streptococcus pyogenes* coined by Friedrich Julius Rosenbach in 1884[34;36].

Streptococci were first classified at the turn of the 20th Century according to their differential capacity to induce haemolysis on blood agar[35]. Pioneering work by Rebecca Lancefield during the 1930s proposed a serological classification scheme based on group-specific polysaccharides[35;37]. She further subdivided group A streptococci according to the M protein found on the cell wall, an important virulence factor against which protective antibodies are formed[34]. Research undertaken during the 1920s and 1930s also identified toxins (streptococcal pyrogenic exotoxins) produced by streptococci as having an important role in the pathogenesis of scarlet fever[35].

2.1.2. Carriage and transmission of *S. pyogenes*

Streptococcus pyogenes is commonly carried in the oropharynx and on intact skin of humans. The genital tract and perianal area are also sites of carriage. Carriage rates vary according to geographical location, climatic factors, season and age[34]. Estimates of pharyngeal carriage range from 12-23% in school-aged children [38;39]. *S. pyogenes* can also contaminate the environment immediately around carriers and those with disease[40]. Different M-types are known to favour mucosal versus cutaneous sites, the latter constituting the higher-numbered types in reflection of their more recent identification[34]. There is some evidence that some serotypes have more pathogenic potential than others[41].

Transmission of *S. pyogenes* is usually through direct contact with droplets of saliva or nasal secretions from carriers or persons with clinical infection, or through skin contact, especially contact with infected lesions. Seminal work carried out at the Warren Air Force base in Wyoming (USA) found transmission rates to be higher in symptomatic than asymptomatic individuals, from individuals carrying the organism in their nose than throat, and from those heavily colonised[42]. Transmission rates have also been found to be increased by crowding[34;42]. The length of incubation is usually fairly short, usually 1-3 days[43]. The period of communicability is typically 10-21 days in untreated individuals with uncomplicated infection. This is significantly reduced once antibiotic treatment has commenced[34;43], with less than 20% of children in one study found to have a positive throat swab 24 hours after commencement of treatment[44].

2.1.3. Diseases caused by *S. pyogenes*

A wide range of clinical infections are recognised as being caused by *Streptococcus pyogenes*, including respiratory, cutaneous, soft tissue and systemic infections. Suppurative presentations commonly associated with this organism are listed in Table 1, with most being potentially caused by a range of different pathogens[34]. The two most important non-suppurative presentations are rheumatic fever and glomerulonephritis.

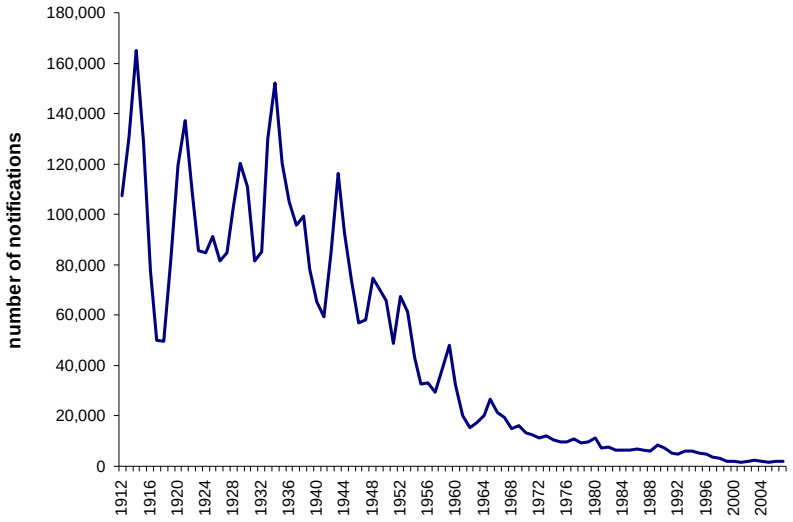
Table 1 **Suppurative infections caused by *S. pyogenes***

Non-focal bacteraemia	
Skin and soft tissue infections	
Cellulitis	Impetigo
Erysipelas	Necrotizing fasciitis
Carditis	
Meningitis	
Upper and lower respiratory tract infections	
Epiglottitis	Pharyngitis
Empyema	Scarlet fever
Mastoiditis	Tonsillitis
Otitis	Pneumonia
Bone and joint infections	
Abdominal infections	
Peritonitis	Appendicitis
Pelvic and obstetric infections	
Puerperal sepsis	Vaginitis

Puerperal sepsis or 'childbed fever' has represented one the most important of these disease manifestations in developed countries during the past three centuries. The introduction of 'lying-in' hospitals in the 17th and 18th Centuries across Europe and the subsequent shift from home birth to hospital delivery provided ideal conditions for the spread of infection[45-47]. High rates of maternal death began to be reported across Europe, with a staggering 1 in 5 maternities in one hospital in Stockholm resulting in death[48]. Seminal findings by Semmelweis in the 1840s demonstrated that the disease was spread through contagious particles carried on hands and overalls of attending clinicians[49]. It took a further 30 years however before this became accepted and infection control measures instigated following the identification of streptococci in clinical specimens by Billroth and Pasteur during the 1870s[48;50].

Development of a mechanism to identify and classify *Streptococcus pyogenes* during the early part of the 20th Century paved the way for the epidemiological study of important disease manifestations: scarlet fever, acute rheumatic fever and puerperal sepsis. Incidence and severity of these diseases fell dramatically over the past century. Recognition of the causative organisms and factors facilitating transmission are likely to have influenced the changing incidence of infection, along with development of antibacterial drugs for use in treatment and prophylaxis during the 1940s[35]. However, incidence of these infections appears to have been falling prior to these developments (Figure 1, HPA), suggestive that other host or pathogen factors may have been important in reducing transmission or infection, for example improved living conditions and general health, or a possible diminution in strain virulence.

Figure 1 **Notifications of scarlet fever in England and Wales, 1912-2007**



After a century of declining incidence of rheumatic fever, reports began to emerge from the USA suggesting a resurgence of disease in military and civilian populations during 1980s[51]. Whilst a resurgence in rheumatic fever in other countries was not generally documented, widespread increases in incidence of invasive disease began to be reported from the 1980s onwards (see **2.4. The burden of severe *S. pyogenes* infection at the turn of the millennium**).

2.2. Measuring and monitoring the incidence of severe *S. pyogenes* infection

Our understanding of the epidemiology of severe diseases caused by *S. pyogenes* is relatively poor compared to many other infectious diseases. Many countries with established infectious disease surveillance programmes have undertaken relatively little surveillance of diseases caused by *S. pyogenes* and other streptococci. However, many are now expanding or modifying their surveillance programmes to capture information on diagnoses of severe *S. pyogenes* infection, not least in light of recent worrying trends in incidence.

Two distinct approaches are commonly used to obtain data of value in understanding and characterising the spread of infections in a population. The most common, pragmatic and economical approach is to find an existing source of information on the diseases of interest, such as point of contact with healthcare services, and establish a mechanism to capture relevant information from this source. In contrast to this opportunistic approach is the establishment of a customised system to capture data not available through existing sources. The advantage of the first approach is that it clearly requires less resource than the second, either from central co-ordinators or from local data providers. This approach is limited, however, to diseases that lead patients to seek contact with healthcare services and to information routinely captured.

To fully understand the epidemiology of diseases caused by *S. pyogenes*, an understanding of transmission dynamics are needed, in terms of how this organism spreads, host and strain characteristics of importance to onward transmission and disease severity and inter- and intra-species competition for ecological niches. A comprehensive investigation following a very large healthy cohort for a substantial period of time would have to be undertaken to explore these dynamics, given the rarity of some of the severe presentations one would be trying to capture. Although this would reveal a host of new information on transmission and incidence, an important first step towards identifying effective prevention strategies, clearly this would be prohibitively expensive. As such, studies have tended to focus on specific elements of disease transmission and incidence.

2.2.1. Statutory requirements for the notification of *S. pyogenes* diseases

Very few countries within Europe list severe *S. pyogenes* infection among their notifiable diseases. In Norway cases of invasive *S. pyogenes* infection have been notifiable since 1975, and all severe infections (including isolates from non-sterile sites, when accompanied by severe clinical presentation) since 1995[52]. Finland similarly made *S. pyogenes* bacteraemia notifiable in 1995, as have Ireland in 2003 and Sweden in 2004 [53-55]. With the exception of these countries, surveillance activities have been predominantly reliant on voluntary reporting systems. There is no current requirement

for national public health institutes to report cases of severe *S. pyogenes* disease to ECDC[56;57].

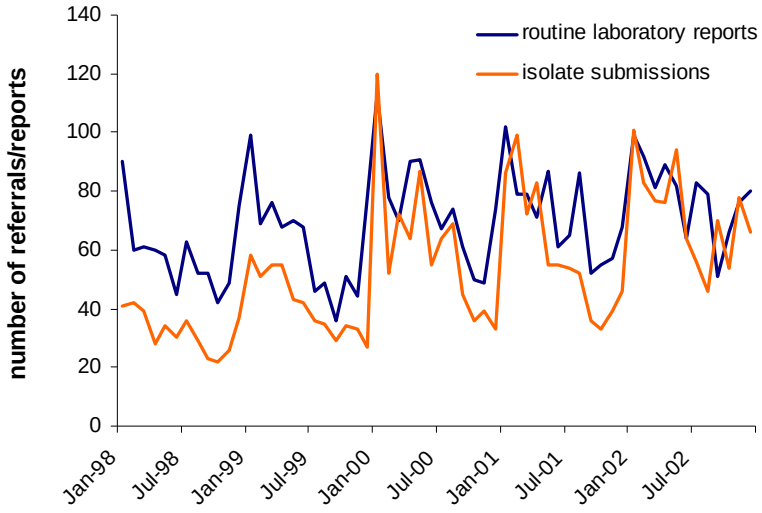
2.2.2. Surveillance methodology

An international network encompassing countries bordering the Arctic (International Circumpolar Surveillance) has been in operation for a number of years, although capture of data on invasive *S. pyogenes* infections has been limited to the USA, Canada and Greenland[58]. In the absence of a dedicated Europe-wide surveillance network, different European countries have been undertaking surveillance of *S. pyogenes* infections according to their own criteria. Common methodological approaches have been adopted between countries, allowing some degree of comparability of results. National or multi-site surveillance activity results identified from the WHO European Region are given in Table 2 (based on Lamagni et al[31], with additional data) and from other WHO regions combined in Table 3. Most operate through the capture of routine local microbiological diagnoses into a central data bank. The quality of data available through such systems has been variable, both in terms of the breadth of information collected and completeness of reporting. Many such systems do not routinely capture clinical information, which is a particular shortfall for *S. pyogenes* infection given the plethora of associated conditions and their differing risk factors.

As many industrialised countries have a recognised national reference centre for microbiological identification and typing of streptococci, surveillance activities have commonly utilised isolate submission for surveillance purposes. This provides information on microbiological characteristics of strains circulating within these countries, such as serotype (based on T and M proteins), sequence typing of the *emm* gene (*emm*ST) and antibiotic susceptibility. A potential drawback can be referral bias, depending on which criteria are applied by laboratories in selecting isolates for referral or by reference laboratories in inviting isolate submission. Isolates that are sent primarily for “epidemiological purposes”, usually referring to the determination of strain relatedness for outbreak control purposes, will be unlikely to represent the primarily sporadic bulk of invasive *S. pyogenes* infections. Referral on the basis of atypical microbiological characteristics or clinical features would also present a biased group of isolates. However, some countries have attempted to circumvent these problems of biased sampling by requesting submission of all invasive isolates, and as such referral

data may provide some important markers of trends in infection (Figure 2, modified from Efstratiou et al [59]). Many countries in Europe have utilised both isolate referral-based and laboratory report-based surveillance systems in parallel (Tables 2, 3).

Figure 2 **Sterile site referrals and laboratory reports of *S. pyogenes* infection, England and Wales 1998-2002**



Sources of information used for routine surveillance purposes have been periodically supplemented through invoking a period of "enhanced surveillance", primarily to gain additional patient, clinical, microbiological and outcome measures. Aside form the countries who participated in the Strep-EURO programme (see **4. Materials and methods**), Belgium also initiated enhanced surveillance in 2004 following an observed sudden increase in invasive *S. pyogenes* disease cases detected through their laboratory surveillance system[60].

2.3. Definitions of severe *S. pyogenes* infection

2.3.1. Definitions applied in different countries

Definitions of 'invasive' or 'severe' *S. pyogenes* infections applied in different countries for surveillance purposes have varied to a considerable degree, with no agreed consensus definition existing. Although ECDC has drafted and ratified among its member states case definitions for many infectious diseases, and is now collating surveillance data on them, *S. pyogenes* infections are not included as presumably not considered a sufficient priority[56;57]. The classification of *S. pyogenes* diseases put forward by a USA working group in 1993 has been adopted by several countries for surveillance or research purposes[24]. The classification divides these diseases according to five groups (Table 4, modified from [24])

Table 4 **Classification of streptococcal infections**

I. Streptococcal toxic shock syndrome
II. Other invasive infections: isolation of *S. pyogenes* from a normally sterile site in patients not meeting the criterion for STSS
    A. Bacteraemia with no identified focus
    B. Focal infections with or without bacteraemia (included meningitis, peritonitis, pneumonia, puerperal sepsis, osteomyelitis, septic arthritis, necrotizing fasciitis, surgical wound infections, erysipelas, cellulitis)
III. Scarlet fever
IV. Non-invasive infections
    A. Mucous membrane
    B. Cutaneous
V. Nonsuppurative sequelae
    A. Acute rheumatic fever
    B. Acute glomerulonephritis

The USA Working Group further divided STSS into *definite* and *probable* according to whether a sterile site isolate was obtained or not, respectively. Categorising STSS as a separate condition introduces a degree of overlap between the above categories, especially with group II, and precludes the possibility of describing the primary presentations of patients with STSS given the primacy of this presentation in their

Table 2 **Invasive group A streptococcal infection surveillance in Europe, 1990 to 2003**

	surveillance methods				latest estimates		references
	surveillance period*	coverage	surveillance method/s	clinical information available	incidence per 100 000 (year)	macrolide resistance (year)	
Belgium	1994-	national	Microbiology laboratory reports	No	1.0 (2003)	-	[61;62]
	1994-	national	Isolates submitted to reference laboratory	No	-	9% (1997)	
Czech Republic	1994-98	national	Isolates submitted to reference laboratory	Yes	0.4 (1994-96)	-	[28]
Denmark	1969-	national	Isolates submitted to reference laboratory	Yes	3.3 (1998)	2% (2003)	[58;63;64]
	2002-	Greenland	Microbiology laboratory reports	Yes	0 (2002)	-	
Finland	1988-96	national	Isolates submitted to reference laboratory	No	-	5% (1996)†	[65;66]
	1995-	national	Microbiology laboratory notifications + isolates submitted to reference laboratory	No	2.3 (2003)†	-	
France	1998-	national	Isolates submitted to reference laboratory (invasive & non invasive GAS)	Yes	-	23% (2002)†	[67-70]
	1987-	national	Microbiology laboratory reports	No	1.7(2002)†	-	
Germany	1996-02	national	Isolates submitted to reference laboratory	Yes	0.1 (1997-02)	-	[71]
Hungary	1975-	national	Microbiology laboratory reports (invasive & non invasive GAS)	No	1.3 (2002)†	-	[72]
Iceland	1975-	national	Microbiology laboratory reports	No	3.8 (1996-02)	-	[73]

Israel	1997-98	national	Isolates submitted to reference laboratory	Yes	3.7 (1997-98)	-	[74]
	1980-	regional	Clinical & microbiology laboratory reports	Yes	4.8 (1990-94)	2% (1987-94)	[75]
Italy	1993-	national	Microbiology laboratory reports (invasive & non invasive GAS)	Yes	0.1 (1994-96)	32% (1994-96)	[27]
Netherlands	1992-03	national	Isolates submitted to reference laboratory	Yes	3.1 (2002)	-	[76]
Norway	1975-	national	Notification through laboratory	Yes	3.3 (2002)	2% (1988-03)	[77;78]
Poland	1997-05	sentinel sites	Isolates submitted to reference laboratory	Yes	-	10% (1997-05)	[79]
Portugal	1998-99	sentinel sites	Isolates submitted to reference laboratory	No	-	11% (1998-99)	[80]
Russia	2000-01	sentinel sites	Isolates submitted to reference laboratory (invasive & non invasive GAS)	Yes	-	11% (2000-01)	[81]
Sweden	1989-	national	Microbiology laboratory reports + isolates submitted to reference laboratory	Yes	2.9 (2000)	-	[8;82;83]
United Kingdom	1975-	England, Wales, Northern Ireland	Microbiology laboratory reports	No	3.5 (2003)[†]	4% (2003)	[84-87]
	1988 -	Scotland	Microbiology laboratory reports	No	3.6 (2002)[†]	-	
	1980 -	national	Isolates submitted to reference laboratory	Yes	-	5% (1994-97)	

* - indicates still ongoing; [†] blood ± CSF only; - not available/applicable

Table 3 **Invasive group A streptococcal infection surveillance outside Europe, 1990 to 2003**

	surveillance methods				latest estimates		references
	surveillance period*	coverage	surveillance method/s	clinical information available	incidence per 100 000 (year)	macrolide resistance (year)	
Australia	2002-04	Victoria	Microbiology laboratory reports	Yes	2.7 (2002-04)	4% (2002-04)	[2;88]
	1996-01	North Queensland	Isolates submitted to reference laboratory	Yes	10.1 non-indigenous & 82.5 indigenous pop (1996-01)	3% (1996-01)	
Canada	2000-	national	Microbiology laboratory notifications	No	3.2 (2003)	-	[3;4;58;89-91]
	1993-	national	Isolates submitted to reference laboratory	No	-	11% (2004)	
	1995-	Montreal	Microbiology laboratory notifications	Yes	2.4 (2001)	-	
	1992-93	Ontario	Microbiology laboratory reports	Yes	1.7 (1993)	-	
	2000-	Arctic	Microbiology laboratory reports	Yes	4 (2002)	-	
Fiji[†]	2000-05	national	Microbiology laboratory diagnoses	Yes	11.6 (2000-05)	-	[92]
Hong Kong	1995-98	sentinel sites	Isolates identified by hospital laboratories (invasive & non invasive GAS)	Yes	-	32% (1995-98)	[93]
Japan	1992-	sentinel sites	Microbiology laboratory reports	Yes	-	4% (1993-2003)	[94-96]

Kenya[†]	1998-02	Kilifi district	Microbiology laboratory diagnoses	Yes	29 in <5y (1998-02)	-	[97]
Mexico	1991-2000	sentinel sites	Isolates identified by hospital laboratories (invasive & non invasive GAS	Yes	-	-	[98]
New Zealand	2001	national	Isolates submitted to reference laboratory (invasive & non invasive GAS	No	-	<1% (2001)	[99;100]
	2003	national	Microbiology laboratory reports	Yes	-	-	
USA	1994-95	Atlanta	Microbiology laboratory reports + isolates submitted to reference laboratory	Yes	5.2 (1994-95)	-	[1;101;102]
	1995-	sentinel sites	Microbiology laboratory reports	Yes	3.9 (2003)	-	
	1999-	Alaska	Microbiology laboratory reports	Yes	3.5 (2003)	-	

* - indicates still ongoing [†] single-site studies included on the basis that the hospital covered the entire country/district population

hierarchical classification. As such, and in consideration of STSS as a complication of streptococcal infection rather than a primary manifestation, many studies have classified STSS as a separate dimension across all clinical presentations.

Adopting a clinically-based case definition for ongoing surveillance purposes is clearly more labour intensive than a definition based on microbiological results alone, and consequently adopted by few countries. Many countries have tended to opt for a simple definition for surveillance purposes – isolation of *S. pyogenes* from blood and other sterile sites. Two notable exceptions are Belgium, where isolates from deep ear sites were included in their routine case definition, and Australia (Victoria) where patients with pharyngeal isolates hospitalised for the treatment of quinsy were included.

2.3.2. Impact of differences in case definition

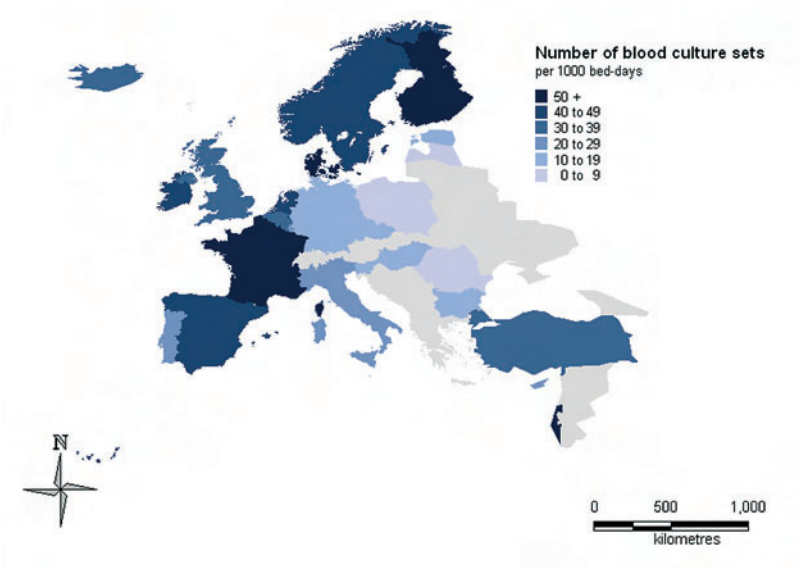
In comparing estimates of the overall burden of severe *S. pyogenes* disease between countries, it is important to take into account the different case definitions used for surveillance purposes. Whereas routinely available data from the UK, Finland and France are based on blood culture isolates only (+/- CSF), most other countries monitor all sterile site isolates. The majority of severe *S. pyogenes* infections result in bacteraemia, however, non-disseminated invasive infections have been found to account for around 10% of cases[6;73;103], although estimates as high as 24% have been documented[104]. This would therefore in part account for the differences in rates observed (Tables 2, 3). Some countries, such as Norway, also monitor cases where the clinical presentation indicates a severe infection but without a sterile site isolate being obtained, increasing case numbers by around a quarter[52]. Surveillance in Belgium, which includes deep ear sites, adds approximately two-thirds more cases[57], although data for sterile site isolates are also available separately, as given in Table 2[61].

2.3.3. Sensitivity and specificity of surveillance systems

Three key factors determine the sensitivity of national *S. pyogenes* surveillance systems 1) failure to take a clinical sample at all, or pre initiation of antibiotic therapy 2) failure by the laboratory in correctly identifying the causative organism 3) failure to report the laboratory result (or refer the isolate) to the co-ordinating centre. A further factor which may in the future impact on the sensitivity of microbiology-laboratory based surveillance is the use of near patient testing, should ward-based kits become available for analysis of blood cultures[105].

As an organism which is fairly easily identified by laboratories, specificity of laboratory results tends to be high, with results from the 2006/07 international external quality assessment scheme indicating 99% correct identification (1640 participants across 37 countries)[106]. The biggest determinants of loss of sensitivity of surveillance systems come from either a failure to take clinical specimens or from a failure to report results to the co-ordinating centre. With regard to the first of these, data illustrating the different thresholds or algorithms applied in clinical settings in different countries are difficult to come by. Anecdotal reports suggest that in the resource-poorer countries within Europe, primarily those in the south, central and eastern Europe, clinical specimens are taken less commonly than among the wealthier nations, with patients being treated empirically on the basis of their symptomatology. This is supported to a degree by data submitted from the participants of the European Antimicrobial Resistance Surveillance System (EARSS) on the rate of blood culture sets taken per 1000 bed-days (Figure 3, based on EARSS annual report 2006[107]).

Figure 3 **Country-specific rates of blood culture sets taken per 1000 bed-days reported by laboratories participating in EARSS, 2004**



Loss of sensitivity due to under-reporting of laboratory diagnoses can be difficult to quantify unless independent sources exist to cross-validate. In many countries coverage is known to be less than complete. In some countries, such as the UK, coverage is very good although under-reporting by active laboratories is known to occur[108]. Studies which capture data from multiple sources allow us to evaluate the sensitivity of these systems. For example, within the UK enhanced surveillance in 2000/01 of invasive *Streptococcus agalactiae* (Lancefield group B) disease captured and reconciled reference laboratory data and routine surveillance data with clinical (paediatrician) reports. This identified an overall sensitivity of laboratory reporting (isolate referral and routine reporting combined) of 83%. The degree of overlap between different sources can be used to perform a capture-recapture analysis to evaluate the true measure of incidence of a given disease[109]. However, unless the data sources are truly independent, this can easily overinflate estimates.

2.4. The burden of severe *S. pyogenes* infection at the turn of the millennium

A recent review commissioned by WHO, published in 2005, estimated that approximately 660,000 cases of invasive *S. pyogenes* disease occur globally each year[110]. Measurement of incident cases has tended to be of the extent of most countries estimation of the burden of severe *S. pyogenes* disease. Although such measures are of vital importance in detecting widespread changes, and reasonable for measuring the burden of acute diseases, they fail to provide a true measure of the burden of infection, which would require measuring the impact to the individuals, their families and to wider society. Few studies have looked at the physical or psychological impact of these infections[111;112], and as such our evaluation of the burden of these diseases remains incomplete.

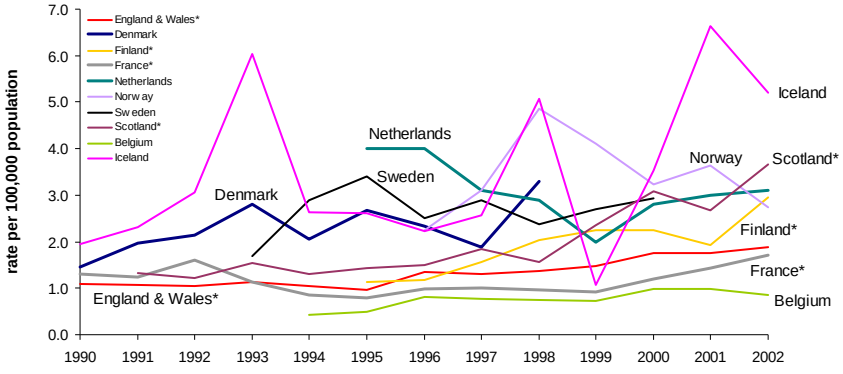
2.4.1. Estimates of overall disease incidence in Europe

Some interesting parallels emerge when comparing results from surveillance activities across Europe over the last decade. Surveillance data from countries who have published five or more consecutive years' results are shown in Figure 4. Results from these primarily northern European countries show some interesting and not entirely uniform trends, although most exited the 1990s with higher rates of disease than they entered the decade. Data from the Netherlands in particular contrast that from other countries, with rates of invasive *S. pyogenes* halving between 1995 (4.0 per 100,000) and 1999 (2.0/100,000), although an upturn was observed subsequently[113]. Most other countries showed reasonably consistent findings suggestive of an overall increase in incidence during the 1990s and into the 2000s, although trend patterns varied markedly from near linear to marked peaks and troughs. Data from Scotland are among the most compelling, showing marked year-on-year rises from the mid 1990s onwards when rates of *S. pyogenes* bacteraemia rose from 1.5/100,000 in 1996 to 3.7/100,000 in 2002, averaging at a 41% increase per year[85;86]. A more diluted rise in reports was also seen in England and Wales throughout the 1990s[114]. Data from Finland also showed a similar pattern, rising sharply from 1996 (1.2) to 2002 (3.0)[66]. Surveillance data from neighbouring Sweden (1993-1997) showed a less clear-cut pattern, rising sharply between 1993 and 1996 before dropping back again to a more stable annual

rate between 2.3 - 2.9/100,000[8;83]. Following the initial reports of a marked rise in severe clinical manifestations of *S. pyogenes* infection in the mid-1980s[15], rates in Norway showed an initial fall only to re-escalate around 1993[52]. More recent web-published data indicate further sharp rises in rates of invasive *S. pyogenes* in Norway between 1996 and 1999 when rates of reports more than doubled to reach 4.9/100,000[77]. Even more pronounced changes are apparent in Iceland, the smallest of the countries examined, where rates of invasive *S. pyogenes* swung from lows of around 1-2 per 100,000 to peaks above 6/100,000, the highest rates observed in any European country over this period. Published data from Denmark showed further increases in the early 1990s to those identified towards the end of the previous decade[16;115], with the latest estimate from this period standing at 3.3/100,000[64].

Surveillance data from France from the early 1990s were suggestive of a downward trend in invasive *S. pyogenes* infections, although recent reports indicate a rise between 1999 and 2002[68]. Annual reporting rates in Belgium showed marked rises between 1994 and 2000[61], from less than 0.5 invasive *S. pyogenes* cases per 100,000 in the mid 1990s to approaching 1/100,000 in early 2000s.

Figure 4 **Country-specific annual rates of invasive *S. pyogenes* infection in Europe, 1990-2002**



* blood (+/- CSF) only

It is of interest to note the pattern of the changes in rates of invasive *S. pyogenes* reports within countries. Whereas countries such as Norway, Sweden, Iceland and the Netherlands showed quite marked up- and downswings in their rates of disease, other countries such as the UK and France have not seen such marked changes. It may be that outbreaks of invasive *S. pyogenes* disease are masked in national data given the larger population size of these countries, although population density is also likely to be an important factor in determining spread. Regional analyses and further microbiological characterisation of isolates would need to be undertaken to confirm if this is the case. Regardless of the patterns of invasive *S. pyogenes* disease, there is general suggestion of increasing rates of infection across Europe since the beginning of the 1990s.

The estimates of disease incidence will of course be influenced by a number of artefactual factors, including the choice of case definition as discussed in **2.3.2. Impact of differences in case definition** and coverage of the surveillance systems, discussed in **2.3.3. Sensitivity and specificity of surveillance systems**. Even with these factors borne in mind, there is a fair degree of variation between countries over this period, with rates of *S. pyogenes* bacteraemia between 1.3 and 3.6/100,000, and rates of invasive *S. pyogenes* (all sterile sites) infection between 0 and 4.8/100,000. To what extent these lower estimates reflect true differences in incidence or other methodological factors, is unclear.

2.4.2. Estimates of overall disease incidence outside Europe

Of the developed countries outside Europe, surveillance data estimating incidence of infection have been published from Australia, Canada and the USA. Estimates of severe *S. pyogenes* disease incidence from Australia and the USA have been broadly similar to those from within Europe during the early 2000s, around 3-4 per 100,000[1;2]. Data from the USA Active Bacterial Core system offer a longitudinal picture, which shows no clear trend pattern during the early 2000s, fluctuating between 3.2 and 3.9/100,000[1]. Disease estimates from eastern Canada (Ontario, Québec) have been similar to those from the USA, although interestingly those from western Canada (Alberta) have tended to yield higher rates up to 5.7/100,000[3;4;90]. This may be explainable through differential participation in surveillance across Canada, although it could conceivably reflect a true difference in incidence, for example due to the climatic differences between the more dry and arid west than the east. Of the less developed

countries, disease incidence estimates have been published for Fiji and Kenya, the latter restricted to children, both of which have indicated high rates of infection, 12/100,000 in Fiji and 29/100,000 among <5 year olds in Kenya[92;97].

2.4.3. Overview of trends within and outside Europe

Data from within Europe has tended to show more consistent trends indicative of an increase in incidence during the 1990s and 2000s[31]. Outside of Europe, most of the longitudinal data has come from the USA, which show relative stability over this period, although reporting rates similar or even slightly higher than reported by European countries[1;116;117]. Reports outside of the USA have tended to indicate increases in rates of disease during the 1990s[4;88].

It is somewhat unclear what the key drivers are for the increases noted by most countries. Discounting artefactual causes, there are several possible hypotheses, including a) an increased circulation of more pathogenic serotypes b) an increase in virulence of the (same) circulating serotypes c) an increase in carriage due to decreased antibiotic prescribing d) an increase in transmission due to behavioural/sociological changes affecting mixing patterns or crowding e) an increased susceptibility due to environmental changes f) a decrease in immunological protection due to lower transmission rates in childhood.

2.4.4. Mortality associated with *S. pyogenes* infection

An total of 163,000 deaths attributable to invasive *S. pyogenes* disease have been estimated to occur each year globally, ranking ninth among infectious causes of death[110]. Between 47 and 77,000 deaths annually have been estimated to be due to maternal sepsis[46;118], representing between 8 and 12% of all maternal deaths in developing countries, and 2% in developed[118]. Of these maternal sepsis deaths, a sizeable proportion are likely to be due to *S. pyogenes*.

Case fatality rate measurements have varied substantially between studies, ranging from 7-23% of patients[2-8]. These differences are due in part to the way this is measured, for example the post-infection follow-up period or whether cause-specific or all cause mortality is measured. More fundamentally, overall measures of mortality will be highly dependent on the different types of disease presentation, serotypes

responsible and the patient groups affected, with young adults tending to have the better outcome than children and the elderly, and those with necrotizing fasciitis (15-45%), pneumonia (20-38%) or who develop STSS (35-81%) the poorest survival[4;6;8;11;89;116;119;120]. Measurement biases can also be introduced where this information is captured by questionnaire survey, as the cases with the worst prognosis are less likely to be missed by clinicians or less likely to be noted as having an uncertain outcome. Using external sources of information on mortality, such as death registrations linked to the patient records, provides a more robust means of measuring case fatality rates. Where the cases represent all those occurring in a given population, these linked deaths can also be translated into a population measure of mortality (deaths per 100,000 population), providing a useful comparator to other competing causes of death[110].

2.4.5. Microbiological characteristics of pathogenic strains

S. pyogenes isolates are typically characterised according to their cell wall T- and M-proteins, with the latter offering greater 'discriminatory power' over the T-protein. Typing schemes are based on either serological identification of the T- and M-proteins, or more commonly genotyping of the *emm* gene which encodes the M-protein.

Within any given population at any given time, an array of different *emm*/M-types are found to circulate, determined by competition between strains in combination with immunological memory of susceptible hosts. The diverse range of *emm*/M-types is in itself a likely reflection of an evolutionary strategy to evade the host immune response, with novel types continuing to emerge. Some strains appear to be more successful given their relatively high frequency in diverse populations, namely M1, M3, M28, M12, M87, M89. M1 and M3 have long since been associated with particularly severe infections such as necrotizing fasciitis, with influxes in either strain being associated with a general increase in incidence of infection and associated mortality[15;20;121]. It is unclear whether these serotypes are equally successful as colonisers and the relative high frequency of severe disease caused by them is a straightforward reflection of their frequency as colonisers at any given time as few robust studies have looked at this. Many studies have compared the frequency of *emm*/M-types causing superficial and severe infections in given population at the same time[8;93;122-124], although these tend to be based on collections of referred specimens which may well not reflect the full

array of either all superficial infections or colonising strains. One study which did obtain a representative sample of carriage, pharyngitic and invasive strains was carried out in Australia, and found that *emm*1 strains were significantly associated with pathogenic potential, and *emm*12 were significantly less pathogenic[41].

Although *S. pyogenes* isolates have remained sensitive to penicillin[125], continued monitoring of penicillin resistance is essential, given the evolution of resistance in other members of the genus which share the same ecological niche. Resistance to other important therapeutic agents, notably clindamycin and erythromycin (see **2.6 Current treatment strategies**), has been observed at low levels in many countries. Although a wealth of published literature on resistance in organisms causing superficial infections have been published, and have formed the basis of international surveillance activities via the SENTRY and PROTEKT schemes[126;127], fewer publications provide estimates for isolates involved in invasive disease, which may conceivably have different resistance patterns to superficial strains. Biases can also be introduced where microbiological sampling occurs after initiation of treatment with antibiotics.

Estimates of macrolide resistance in *S. pyogenes* strains causing invasive disease in Europe during the 1990s and early 2000s showed considerable variation, a possible reflection of differences in macrolide consumption at a population level[128]. Within country changes in macrolide consumption, albeit those that result from variation in prescribing according to seasonal patterns of *S. pyogenes* disease, or as part of longer-term programmes to reduce macrolide consumption, have been shown to decrease erythromycin resistance levels[65;129]. However, the impact on isolates causing invasive disease has not been demonstrated. Most countries in Europe have reported erythromycin resistance in less than 10% of isolates (Table 2). Notable exceptions are in France and Italy where over 20% of isolates were found to be resistant, possibly linked to their high levels of macrolide consumption[128]. Prevalence of erythromycin resistant organisms involved in invasive disease is increasing in some countries[67;130]. Outside Europe, estimates of macrolide resistance in invasive isolates collected during the 1990s and 2000s varied from 0.4% to 11% (Table 3).

Fewer large-scale studies have reported rates of clindamycin resistance. This may be in part due to practical considerations as isolates found to be erythromycin resistant have

to have clindamycin resistance tested alongside erythromycin to check for inducible resistance[131]. A further reason may be the high cost of clindamycin[125]. Of the studies that have reported clindamycin susceptibility, 22% of invasive isolates in Italy 1994-96 were reported as resistant[27], and 12% in a small subset of isolates in Spain[132]. Substantially lower estimates have been published in Poland (5%), Japan (4%), UK and Ireland (1%) and Australia (no resistance)[2;79;96;133]. Data from non-invasive isolates show even greater variability, from 30% in Portugal[80], to 3% or less in Brazil, Bulgaria, Greece, New Zealand, Russia and the USA[81;99;134-137].

2.5. Control of severe *S. pyogenes* infection

In the absence of a licensed vaccine, control measures for severe *S. pyogenes* infection have been reliant on targeted antibiotic prophylaxis and measures to decrease transmission via fomites or person-to-person spread. The design of such measures to prevent primary or secondary cases of severe *S. pyogenes* infection rests on the ability to identify any groups or activities conveying increased risk of infection to target control measures. As infections which are largely sporadic, occur in a broad cross-section of the population and are generally rare, opportunities for primary prevention of severe *S. pyogenes* disease are rather limited.

2.5.1. Factors predisposing to severe *S. pyogenes* infection

Our identification of factors which predispose to severe *S. pyogenes* infection is somewhat curtailed as few studies have applied rigorous epidemiological study methods to examine this, comparing exposures in cases to recruited controls or to normative (population) data. In part, this may be due to the array of different proposed risk factors, as deriving sufficient statistical power to evaluate many of the less common exposures would be difficult as the number of 'exposed' cases will remain small. For this reason, factors such as premature birth, clearly posing a particular risk of severe sepsis, has not been formally evaluated by comparison to a reference group (see Table 5). In contrast, some exposures such as skin trauma are so common place as to require a very large sample of cases and controls to observe any meaningful difference, or comparison to normative data which would be hard to come by. As such, only a handful of factors have been identified by analytical means as conferring heightened risk of infection: age, male sex, ethnicity, HIV infection, varicella, smoking, household crowding, diabetes, heart

disease, injecting drug use, malignancy and pregnancy (Table 5). Most of these risk factors are reasonably non-specific to *S. pyogenes* infection, and their relative importance is likely to change over time as the prevalence of the underlying conditions change in frequency.

Circumstances which breach the skin barrier provide a portal of entry for the organism, albeit traumatic or surgical wounds, chronic infection sites or chickenpox lesions. Injecting drug users are one group at particularly high risk, as a direct result of injection site contamination or indirectly as a result of straitened living circumstances. Many studies have identified men as being at higher risk of severe *S. pyogenes* infection than women, although again this seems to be the case for invasive bacterial infections in general[138;139]. Cases of necrotizing fasciitis are particularly more common in males though[140], possibly a reflection of occupational risk given the importance of skin trauma as a risk factor. Abattoir workers and meat handlers have also been documented as being overrepresented among cases of skin sepsis[141-143]. Whether this increased vulnerability is due to increased risk of skin trauma from working with knives or connected with meat itself is unclear, although other occupational groups at risk of minor trauma do not seem to experience outbreaks of *S. pyogenes* skin sepsis as commonly as meat handlers.

A number of patient factors have also been identified as conferring increased risk of severe *S. pyogenes* infection. These include medical and other conditions affecting immune function, such as diabetes, malignancy and alcoholism. The elderly and to a lesser extent young children have a higher risk of infection than young adults. Interestingly, an exception is seen in surveillance data from Alaska which indicates higher rates in children (<2y; 35/100,000) than the elderly (65y+; 14/100,000) among its native population[102]. Rather controversially, use of non-steroidal anti-inflammatory drugs has been linked to development of necrotizing fasciitis although not consistently across all studies[144].

Many countries worldwide have reported a higher incidence of severe *S. pyogenes* disease in particular ethnic groups. Studies from North America have found higher rates of infection in black Americans[116;117], native Americans[6] and aboriginals in the arctic region of Canada[89] compared to white European settlers. From the pacific

regions, indigenous Australians[88] and to a lesser extent Fijians[92] have also been found to be at heightened risk of invasive disease. Although few such studies have been carried out within the European region, in part owing to political sensitivities in some of the more active northern countries, two studies from Israel report high rates of infection in its Bedouin and Jewish population compared to estimates from western Europe[74;75]. Given the disparate nature of the populations found to be at heightened risk, it may be that different factors explain the increased susceptibility in different groups. Darkly pigmented skin prevents the synthesis of calciferol (vitamin D) and as such dark skinned individuals residing in non native areas with less intense UV light exposure are more prone to diseases linked to vitamin D deficiency[145]. Given the connection between vitamin D and immune function[146], this could in part provide an explanation for the increased rates of infections in black Americans. Non genetic factors could also play a role, given that customs, living conditions, prevalence of diabetes and alcoholism differentiate these populations from white Europeans.

Of the risks posed to women, childbirth is amongst the highest as a result of bacteria colonising the vagina contaminating traumatic wounds incurred during the birthing process. Ascension of these bacteria into the uterine sac following rupture of membranes presents a further risk to the unborn child[147].

Given the characteristic transmission dynamics of *S. pyogenes*, most severe infections occur sporadically, with the affected individual having no close contact with another individual with superficial or severe disease; the organism is presumably transmitted from an asymptomatic carrier. Close contact with individuals symptomatic of superficial infection, such as pharyngitis, have been shown to increase risk of severe infection[148;149], as has close contact with individuals with severe disease, albeit an uncommon occurrence[89;150-152].

Table 5 **Factors identified as potentially predisposing severe *S. pyogenes* infection**

risk factor	**level of evidence**			**studies**
	descriptive*	population comparison	case-control	
Demographic factors				
Age (infants & elderly)	x	x		[1-4;6;8;15;90-92;101;102;116;117;130]
Male sex		x		[3;90;116;130]
Ethnicity~				
African American		x		[101;116;117]
Bedouin population		x		[75]
Canadian Arctic aboriginal		x		[58]
East African		x		[97]
Jewish		x		[74]
Native American		x		[6]
Native Alaskan		x		[102]
Indigenous Australian		x		[88;153]
Pacific Islanders		x		[92]
Underlying conditions				
Alcoholism	x			[1-3;6;7;9;10;79;91;101;102;104;116;117;154;155]
Benign tumour	x			[75]
Chronic respiratory conditions	x			[1-3;6;7;91;101;102;116;117;120;132]
Chronic & traumatic skin lesions	x			[1;7;9;27;71;74;75;79;104;116;117;132;154;155]
Congenital abnormalities	x			[9]
Diabetes	x	x	x	[1-3;6;10;27;71;74;91;101;102;104;116;117;120;120;132;149;155] [9]
Endocrine disorders	x			[2]
Epilepsy	x			[79]
Gastrointestinal disorders	x			[2]
Glaucoma	x			[2]
Heart disease	x	x	x	[1-3;6;7;9;10;101;116;117;120;132;149;155;156]
Hypertension	x			[71;111;120;157]
Immunosuppression (non-HIV related)	x			[1;2;7;10;27;71;101;104;117;120;132;154;156]
Injecting drug use	x	x	x	[1-3;6;7;10;71;101;104;116;117;132;149]
Liver disease	x			[2;3;10;79;101;117;132]
Malignancy	x	x	x	[1;3;6;7;10;27;74;75;79;91;101;116;117;120;132;149]
Metabolic disorders	x			[2]
Non steroidal anti-inflammatory drug use	x		x	[2;7;27;71;104;104;148]

Neurological & psychological disorder	x			[2;6]
Obesity	x			[101]
Pregnancy & childbirth	x	x		[3;71;74;79;91;101;104;117;158]
Prematurity	x			[75;120]
Renal diseases	x			[1-3;6;79;101;117;120;132]
Rheumatoid arthritis & polymyalgia rheumatica	x			[10;132]
Smoking	x		x	[2;101;102;148;149]
Steroid use	x		x	[6;9;149]
Trisomy	x			[120]
Vascular disease	x			[10;101;119;132;156]
Antecedent /concurrent infection				
Epstein-Barr virus	x			[79]
Herpes zoster	x			[156]
HIV	x	x	x	[2;9;79;91;101;116;117;132;149]
Influenza	x			[156]
Pneumonia	x			[79]
Rotavirus	x			[79]
Scabies	x			[79]
Varicella (children)	x	x		[2;3;6;71;74;75;79;82;101;104;117;119;132;155;159]
Living conditions & socioeconomic factors				
High number of household members			x	[149]
Hypothermia			x	[79]
Low number of rooms in household			x	[148]
Co-habitation with a child			x	[148]
Low income			x	[148]
Living in unhygienic conditions	x			[155]
Malnutrition (children)			x	[75;97]
Ecological factors				
Winter onset		x		[3;15;61;71;75;90;91;116;117;156]

* without formal statistical comparison to any other group

~ compared to populations of European origin

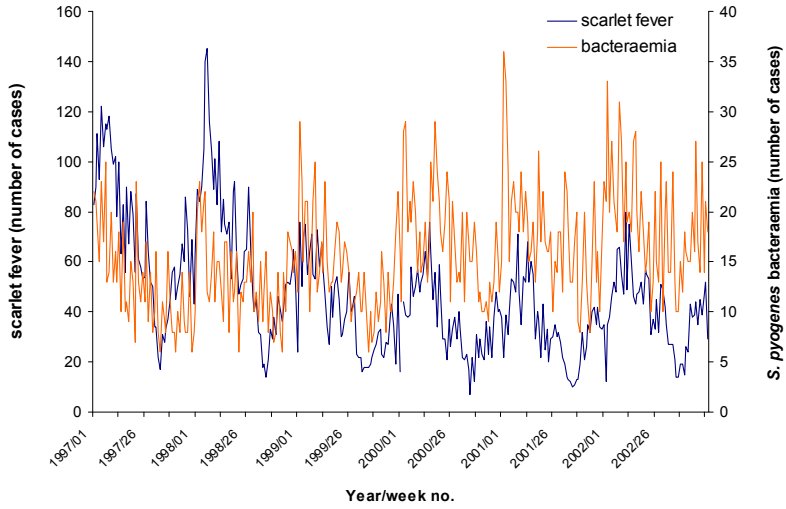
Although a number of risk factors have been identified, as outlined above, one of the distinctive features of severe *S. pyogenes* infections is its ability to affect the otherwise fit and healthy. Between 17-31% cases occur in individuals with no evidence of any particular risk or predisposing factor[16;28;101;117].

2.5.2. Seasonal patterns of infection

One of the defining characteristics of *S. pyogenes* infections are the distinctive seasonal patterns in incidence of disease (Figure 5, HPA). Invasive disease patterns typically have a higher frequency between late Winter and early Spring, and substantially lower frequency in the late Summer/Autumn, a finding fairly ubiquitous across Europe[15;16;76], with few exceptions reported[111]. Similar patterns are also seen for scarlet fever and streptococcal pharyngitis[160;161]. Impetigo tends to have a summer peak, although its aetiology is divided between staphylococci and streptococci, and may be closely tied to insect bites[162]. Outside of Europe, marked and very similar seasonality has been reported from the USA[116;117] and Canada[3;90]. Invasive pneumococcal disease shows a very similar seasonal pattern[163], and interestingly, a broadly similar pattern has been described for *Streptococcus suis* infections in pigs[164].

The explanations behind these seasonal patterns remain rather unclear. Possible explanations include environmental factors, such as impact of climatic changes on mucosal defence barriers or cumulative ultraviolet light exposure on immunological function, or indirectly as a result of seasonal patterns of viral respiratory infections which may induce vulnerability to *S. pyogenes* infection[165]. Other factors which may be of relevance include the influence of weather conditions on social and behavioural patterns, in particular indoor gathering vs outdoor activity, and similarly the impact of the academic term-holiday cycle on transmission patterns. The impact central of heating systems in lowering humidity may also be a contributing factor. Interplay of different factors could explain the changes seen at different times of the year.

Figure 5 **Weekly counts of scarlet fever notifications and *S. pyogenes* bacteraemia diagnoses (all ages), England & Wales 1997-2002**



2.5.3. Control of *S. pyogenes* in community settings

Opportunities for primary prevention of severe *S. pyogenes* infection in community settings are rather limited. As an important risk factor for infection in children, prevention of varicella could represent one of the few opportunities for primary prevention. Estimates from Canada suggest that 10% of paediatric invasive *S. pyogenes* cases could be prevented through universal childhood varicella vaccination[166]. At present, only a limited number of countries in Europe have opted to include varicella vaccination within their childhood immunisation programmes (Austria, Cyprus, Germany, Italy, Spain, Switzerland)[167].

Where resurgences of infection occur in particular population sub-groups, such as injecting drug users, opportunities for targeting advice on prevention or detection of early signs of infections can arise. In outbreak situations, measures to prevent further cases will be dependent on the specific situation and what intelligence is available at the time on which to base decisions, but are likely to include antibiotic prophylaxis to contacts. For example, outbreaks of soft tissue infection have also been associated with military training camps or contact sports, where the close proximity of susceptible hosts coupled by activities which may result in localised skin trauma provide ideal circumstances for

facilitating spread[168]. Under such circumstances, control measures are likely to include decontamination of shared facilities along with advice on not sharing towels and other personal items.

Designing secondary control measures for the prevention of cases in household contacts is rather hampered by a lack of precise data evaluating the risk to household contacts. Obtaining such data would necessitate the identification of all linked cases within a given population at a given time, along with information on how many individuals were at risk in the given household and any particular risk factors among each of these members. As such, estimates are likely to have a wide margin of error given the poor sensitivity of many surveillance systems to identify linked cases and practical difficulties in obtaining information regarding the household constitution. Of the studies which have tried to systematically identify household clusters in a given population over a defined period of time, only 5 household pairs have been identified by the USA and Canada combined and a further 5 in the UK[89;91;151;152]. Given these small sample sizes, estimates of risk to household members are imprecise, although certainly higher than among the general population. Even with this information, a difficult and subjective judgement needs to be made by policy makers regarding the threshold of 'numbers needed to treat' to prevent a case. As a result, different countries have differentially evaluated the risk to household cases from 66 to 294 per 100,000 and consequently have adopted different strategies for secondary control in household settings[169]. In Canada, antibiotic prophylaxis is recommended for all household members where a case of severe *S. pyogenes* infection arises in the community [89] whereas in the USA prophylaxis is only recommended where one or more household members is at high risk of infection[150]. Within Europe, the UK was the first country to publish guidelines on the management of community contacts, with the recommendations for prophylaxis being restricted to mothers and infants where the other develops an infection, or institutional/household settings where 2 or more invasive cases arise[125;152]. Norway, France and Ireland have since published their own guidance[170-172]. The effectiveness of any such measures will be limited however, as clustered cases often arise very close together in time[173], limiting the opportunity for administration of prophylaxis or early treatment. All countries emphasise the importance of providing household members with information on possible early signs of invasive *S. pyogenes*

infection and the course of action to be taken should they arise, which in itself may be as effective a measure as chemoprophylaxis.

2.5.4. Control of *S. pyogenes* in hospital and institutional settings

The foundations of hospital infection control were based on the observations of Semmelweiss that effective hand hygiene and droplet precaution measures could lower the risk of puerperal infections and post-partum mortality[48]. Although this eventually paved the way for the introduction of modern-day hospital hygiene guidelines, it is of interest to note that guidance specific to the control of *S. pyogenes* infections in maternity settings does not exist in many countries[174]. Clusters of invasive *S. pyogenes* disease in maternity settings continue to occur, on occasion resulting in the death of otherwise healthy young women[8;13;52;175].

Guidelines for the control of *S. pyogenes* in hospital settings in general do exist in France, Ireland, Sweden, Canada and the USA, with France making specific recommendations for maternity units[89;150;172;176;177]. Recommended measures for investigating hospital clusters in maternity or other settings range from screening of staff caring for the affected patients to all unit staff and their household members[174].

Outbreaks of severe *S. pyogenes* infection in a range of institutions have been reported, in particular residential facilities providing care for the elderly owing to the vulnerability of these residents[91;178-181], necessitating the use antimicrobial prophylaxis to break the cycle of transmission and/or provide early treatment[182]. A study in Atlanta found that nursing home residents were eight times more at risk than age-matched counterparts residing in the community[101]. Schools, nurseries and other childcare facilities have also been the focus for clusters of invasive disease, especially concomitant to outbreaks of varicella[40;183]. Control measures in such instances can involve vaccination to limit the scale of the varicella outbreak and secondary bacterial infections, alongside antibiotic prophylaxis. Schools can also provide a pool of carriers to seed invasive infections among family contacts, as seen in one outbreak in the USA[38], offering opportunities for chemoprophylaxis of the key reservoir for an outbreak.

2.5.5. Vaccines under development

Given the nature of these infections, vaccination provides a more realistic opportunity for primary prevention, although not without some possible limitations (see **6.1.7 Potential impact of vaccine candidates**). Two vaccines are currently under trial, a hexavalent preparation covering M-types 1, 3, 5, 6, 19, 24 and 26-valent vaccine covering 1, 3, 5, 6, 19, 24, 29, 14, 12, R28, 18, 2, 43, 94, 22, 11, 59, 33, 89, 101, 77, 114, 75, 76, 92[184-186]. Phase II testing of the 26-valent vaccine in adults have shown promising results[185;187], with further test results from children awaited.

2.6. Current treatment strategies

2.6.1. Antibiotic therapy

Given that *S. pyogenes* has to date remained sensitive to penicillin, this remains the first-line treatment of choice once *S. pyogenes* has been identified as the cause of the sepsis[34;188]. Using clindamycin in combination with penicillin G (benzylpenicillin) has been shown to inhibit the activity of virulence factors, lowering the risk of STSS[34]. Where penicillin allergy is reported, cephalosporins or vancomycin can be used as an alternate[188]. Speed of initiation of intravenous antibiotic therapy is essential given the rapidity of the clinical course[9-11]. Delayed treatment as a result of misdiagnosis of key signs of deep seated *S. pyogenes* infection has been associated with increased likelihood of death[13;157;183].

2.6.2. Intravenous polyspecific immunoglobulin G

Whilst there is some evidence that intravenous polyspecific immunoglobulin G (IVIG) may improve the outcome of patients with sepsis and septic shock[189;190], the therapeutic value of its use specifically for the treatment of severe *S. pyogenes* infection remains undemonstrated. An observational study carried out in Canada between 1992 and 1995 measured a decreased 30 day mortality in patients treated with IVIG, although survival was unusually high in the untreated group[191]. A recent randomised controlled trial in Sweden was prematurely stopped owing to a lack of power for the study to evaluate a difference between its control and treatment arms due to low numbers of patients recruited[192]. Despite the lack of robust evidence of efficacy, it remains a recognised adjunct to antibiotic therapy[188;193].

2.6.3. Surgery and other therapeutic approaches

Where tissue necrosis or gangrene is suspected, surgical exploration is essential along with immediate debridement of affected and surrounding tissue, even if likely to render the patient vulnerable to surgical wound infection[34]. Other more novel approaches include use of hyperbaric chambers for the treatment of necrotic infections, which facilitate blood supply to necrotic tissue through the use of hyperbaric oxygen[194].

3. AIMS OF THE STUDY

In light of recent reports indicating global changes in the epidemiology of severe clinical manifestations of *S. pyogenes* infection, and given the disparate and disconnected surveillance activities across Europe, leading microbiologists from across Europe formed a unified network (Strep-EURO) to take forward a programme of work relating to severe *S. pyogenes* disease. The overall aim of this study was to substantially improve our understanding of the epidemiology of severe *S. pyogenes* disease in Europe. This would be achieved through the following objectives:

- To measure and compare the overall and disease-specific burden of *S. pyogenes* disease in eleven countries across Europe
- To identify and compare key risk groups in each country to potentially identify targets for public health intervention
- To identify factors associated with development of STSS as a means of directing future basic research into disease pathogenesis
- To undertake an in-depth analysis of cases occurring in UK injecting drug users according to *emm* types, clustering, clinical presentations and outcome to better understand the possible modes of transmission and burden of disease in this group
- To better understand clinical, demographic, microbiological and other possible predictors of mortality

Pooling of data from the eleven countries would yield a powerful base to examine the interrelation between the host and pathogen factors by providing a large sample size for analysis. This in turn would yield findings of value in directing public health action, as well as directing future research. A particular focus was placed on UK cases given the additional clinical information provided for these cases and the evident differences in the epidemiology of these infections in the UK compared to any other country.

4. MATERIALS AND METHODS

4.1. Overview of the Strep-EURO project

Funded by the Fifth Framework Programme of the European Commission's Directorate-General for Research (QLK2.CT.2002.01398), the Strep-EURO network was launched in September 2002 (www.strep-euro.lu.se). Twelve participants across eleven countries took part in Strep-EURO (two from Sweden), with overall co-ordination provided by the University of Lund in Sweden. The programme of work was divided into seven work packages (lead country given in brackets):

WP1 Establishment of a surveillance and reference laboratory network across EU and Associated Countries (Lund, Sweden)

WP2 Data management (Finland)

WP3 Laboratory diagnostics of GAS (UK)

WP4 Clonal identification of streptococci (Germany)

WP5 Virulence characteristics of invasive GAS strains (Lund, Sweden)

WP6 Susceptibility of streptococcal isolates to antibiotics (Denmark)

WP7 Project management (Lund, Sweden)

This PhD thesis will focus on data generated through the activities in WP1, 2, 3 and 6.

4.2. Surveillance methods

4.2.1. Case definitions

Cases were defined as patients with *S. pyogenes* isolated from a normally sterile site, or non-sterile site in combination with clinical signs of streptococcal toxic shock syndrome (STSS). Within the UK a broader case definition was applied with the inclusion of patients with non-sterile site isolates with one of the following severe presentations (I, III, IV): pneumonia, necrotizing fasciitis, puerperal sepsis, meningitis or septic arthritis. These additional cases were not transferred to the central database but were retained for UK analyses only.

The 1993 Working Group on Severe Streptococcal Infections' definition of STSS was used[24]: hypotensive shock in conjunction with two or more specified clinical indicators (renal impairment, abnormal liver function, respiratory distress, erythematous rash, disseminated intravascular coagulopathy, soft tissue necrosis), with cases of 'definite' (those with sterile site isolates) and 'probable' (those with non sterile site isolates) STSS combined.

4.2.2. Case ascertainment

All participating countries undertook prospective enhanced surveillance between 1st January 2003 and 31st December 2004 to identify cases of severe *S. pyogenes* disease diagnosed during this period. Methods employed to identify cases varied by country; most identified cases through invited submission of isolates from local microbiology laboratories to the national streptococcal reference centre. The majority of participants identified cases from across the country, with some exceptions as noted in Table 6. Three countries (Finland, Sweden and the UK) identified cases using two separate sources to maximise case ascertainment, with cases reconciled to avoid duplicate counting.

Table 6 **Severe *S. pyogenes* infection surveillance methods adopted by Strep-EURO participating countries, 2003-04**

		surveillance network		
	surveillance method(s)	**catchment area**	**sites**	**estimated catchment population in millions** (% total population)
Cyprus	Isolate submission to reference laboratory	National	All hospitals	0.74 (100)
Czech Republic	Isolate submission to reference laboratory	National	Sentinel sites	4.57 (45)
Denmark	Isolate submission to reference laboratory	National	All hospitals	5.39 (100)
Finland	Laboratory notifications +	National	All hospitals	5.23 (100)
	Isolate submission to reference laboratory	National	All hospitals	
France	Isolate submission to reference laboratory	National	All hospitals	61.93 (100)
Germany	Isolate submission to reference laboratory	National	All hospitals	82.53 (100)
Greece	Isolate submission to reference laboratory	Athens Metropolitan area	All hospitals	4.02 (38)
Italy	Isolate submission to reference laboratory	National	All hospitals	57.00 (100)
Romania	Isolate submission to reference laboratory	National	Sentinel sites	21.74 (100)
Sweden	Laboratory notifications +	National	All hospitals	8.99 (100)
	Isolate submission to reference laboratory	National	All hospitals	
UK	Laboratory reports +	England, Wales, N Ireland	All hospitals	54.86 (100)
	Isolate submission to reference laboratory		All hospitals	

4.3. Study data collected

4.3.1. Clinical and risk factor data

A standardised surveillance dataset was defined, comprising demographic, clinical and risk factor information for cases collected through the questionnaire disseminated by each participant. The following items were included in the questionnaire:

- *Country*
- *Unique case number*
- *Sex*
- *Age*
- *Region*
- *Date of sample collection*
- *Specimen site/s*
- *Clinical condition/s:* no focal symptoms, STSS, necrotizing fasciitis, cellulitis, arthritis, puerperal sepsis, meningitis, other
- *Treatment/s and procedures*: admitted to ICU, ventilatory assistance required, surgery performed because of GAS infection, other
- *Additional clinical markers:* hypotensive shock, coagulopathy, renal impairment, liver abnormality, respiratory distress, erythematous rash, soft-tissue necrosis, other
- *Outcome due to GAS infection:* vital status at 7 and 30 days
- *Predisposing factor/s:* diabetes, current injecting drug use, chickenpox, immunosupression, chronic skin lesions/wound, surgical operations within 7 days, hospital acquired infection (hospital care within 48 hours), other
- *Cluster or outbreak identifier*

Additional items were optionally included by some countries (see **Annex 10.1** detailing the UK questionnaire). In Denmark and Sweden, as enhanced surveillance was already in place, existing questionnaires were used with any differences to the study questionnaire items noted for future analysis. Mortality outcome data was obtained through the national deaths registry in Denmark, Finland (2004 only) and the UK, otherwise through the questionnaire. For the UK, mortality information was available

from both the questionnaire and from the deaths registry (obtained from the Office for National Statistics). Information placed on patients' death certificates was available for UK cases (IV), namely date of death, place of death and certified cause/s of death (underlying and contributory causes). The UK questionnaire distinguished deaths according to whether they were attributed to *S. pyogenes* infection (as the main or contributory cause) or not.

In Finland, clinical and risk factor data were collected by an infectious disease clinician's review of patient medical records in a predefined area (Pirkanmaa health district, Western Finland, population of 440,000).

4.3.2. Collection and characterisation of isolates

Isolates were collected by the national reference (or designated central) laboratory for each of the 11 countries. Isolates were characterised according to their M protein using conventional serological methods (M type immunodiffusion and anti-OF typing) or *emm* gene typing (*emm* PCR ELISA, *emm* reverse line blot hybridization or gene sequencing) [195-197]. Antibiotic susceptibility testing was carried out by the central reference laboratory for all countries except the UK, where testing was undertaken by the original referral laboratories according to local standard operating procedures, primarily using disc diffusion. Susceptibility testing by the national reference laboratories was undertaken using E-test, disc diffusion or agar dilution with MICs interpreted against standard breakpoint references (CLSI, SRGA, BSAC and CA-SFM)[198]. Isolates were tested against erythromycin, tetracycline, clindamycin and optionally against linezolid, dalfopristin/quinopristin, telithromycin and moxifloxacin.

Three external quality assessment studies were included within the remit of the Strep-EURO programme, for serological and molecular (*emm*) typing, PFGE subtyping and antimicrobial susceptibility determination using phenotypic and genotypic methods. Results from these demonstrated equivalence of techniques adopted in each country removing the need for further standardisation[195;198].

4.3.3. Collation and validation of study data

Data were formatted and transferred to the data hub in Finland according to a specified protocol, and stored in a secure SQL Server database. Further validation checks were

undertaken on the combined data to identify any logical inconsistencies in the data supplied and to ensure all cases met the case definition. Cases marked as having STSS were checked to ensure this definition was met according to the clinical indicators given, whilst these same indicators were also used to identify cases not marked as STSS but which met the case definition.

4.4. Analysis of project data

All comparative analyses across the Strep-EURO participating countries were based on cases meeting the agreed case definition and using the standardised dataset. Case fatality ratios were based on all cause mortality. Analyses of case fatality made on the UK cases alone were restricted to attributable deaths (main or contributory cause) for papers I and III. For paper IV, analyses were based on all-cause mortality.

Additional cases captured in the UK from the expanded case definition, along with additional data items collected from the UK questionnaire or through linkage of records to the national deaths registry (IV), were not included in comparative analyses.

Data were extracted from the SQL Server and imported into STATA™ software (release 8.2 College Station, Texas: Stata Corporation, 2005) for statistical analysis.

4.4.1. Statistical methods

Descriptive statistics were undertaken, with χ^2 and Kruskal-Wallis tests being applied to test for statistical significance of differences between subgroups in proportions and distributions of continuous variables respectively, and linear regression to evaluate associations between continuous variables (Pearson correlation for binomial data and Spearman's Rank for rates). Rates of infection were calculated using mid-year resident population estimates as denominators for the respective countries for each year (or closest available year) according to age and sex. Exact 95% confidence intervals (CI) around rates and rate ratios were calculated according to the Poisson distribution (I, II), and according to Binomial distribution for proportions (II, IV).

UK cases were linked to death registrations using probabilistic linkage methods (IV) to circumvent any problems of missing or erroneous identifiers within the surveillance

reports[199]. Deaths from all causes occurring up to 30 days after infection were identified from this linkage. Certified cause/s of death (underlying and contributory causes) were analysed according to ICD-10 codes provided and time between diagnosis and death was measured from the date the culture positive specimen was taken (post-mortem cultures were included with those taken on the day of death). Kaplan-Meier survival curves were generated to describe survival from date of diagnosis. Survival between subgroups was compared using a non-proportional test for equality of survivor function (Peto-Peto-Prentice).

Unconditional logistic regression analyses were undertaken to evaluate associations between outcomes of interest and potential explanatory variables (II, III, IV). Models were analysed in a backward step-wise fashion, with removal of non-significant variables at each stage in order of magnitude of the p-value obtained from Likelihood Ratio tests (LRT). All variables with an LRT p value of below 0.2 were kept until the final model, after which only variables with $p<0.05$ were retained.

4.4.2. Standardisation of rates

Overall country rates were age-standardised according to the European Standard Population (II, see **Annex 10.2**). This was undertaken to facilitate comparison of rates between countries whose demographic population structure may vary and in part explain differences in crude rates, given the association between age and risk of infection.

4.4.3. Geographical mapping

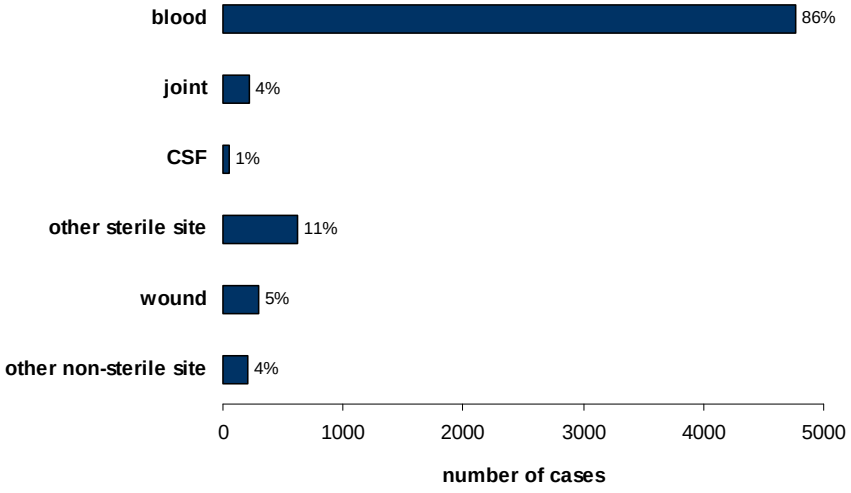
Country-specific rates were mapped using MapInfo Professional© (version 8.0, release build 18 Troy, New York: MapInfo Corporation, 2005). This software was also used to calculate distances between diagnosed cases in the UK to identify clustering, when combined with temporal information (III). Potential clusters were defined as cases occurring with 10km of each other and diagnosed with 30 days.

5. RESULTS

5.1. Overview of cases identified across Europe

During 2003 and 2004, a total of 5522 cases meeting the definition for severe *Streptococcus pyogenes* infection were identified across the eleven participating countries (Table 7). Of these 5522, 5462 (99%) had sterile site isolates submitted, and the remainder included on the basis of having STSS. Eight-six per cent of cases (4771) had a blood culture positive isolate, 4% (224) joint isolates, 1% from CSF (50) and 11% (627) from other sterile sites (Figure 6).

Figure 6 **Site of *S. pyogenes* isolation from cases of severe infection, Europe 2003-04**



The number of cases identified in each country varied from four in Cyprus (0.1% of all cases) to 3630 in the UK (66% of all cases). Considerable variations in age distributions were seen between countries, with no clear geographical pattern. Cases in Greece were primarily drawn from a children's hospital in Athens owing to low participation in other hospitals, resulting in a largely paediatric sample. Excluding Greece, significant variations in age distributions between countries were apparent, (Kruskal-Wallis Test $\chi^2_{(9\ df)}$=261.47; p<0.001), cases being notably older in Sweden (median 70 years) than all other countries (Table 7). Overall, 53% of severe *S. pyogenes* cases were male, with most countries observing slightly higher numbers of cases in men than women, with no significant variation between countries in this ratio ($\chi^2_{(10\ df)}$=17.39; p=0.07).

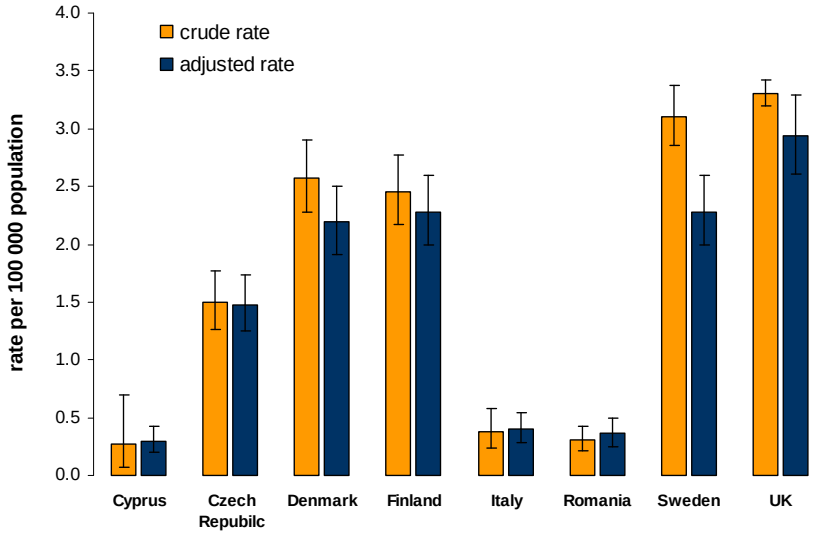
Table 7 **Summary results of severe *S. pyogenes* cases identified in Europe, 2003-04**

	total		**age distribution (years)**						**sex**	
						children (<16y)	**adults** (16-60y)	**adults** (> 60y)	**male**	
	no.	(%)	**mean**	**median**	**range**	no. (%)	no. (%)	no. (%)	no.	(%)
Cyprus	4	(<1%)	20	14	0-53	3 (75%)	1 (25%)	0 (0%)	2	(50%)
Czech Republic	137	(2%)	43	47	0-91	30 (22%)	71 (52%)	36 (26%)	78	(57%)
Denmark	278	(5%)	56	61	0-100	27 (10%)	109 (39%)	142 (51%)	127	(46%)
Finland	257	(5%)	49	53	0-92	20 (8%)	159 (62%)	78 (30%)	139	(54%)
France	272	(5%)	42	42	0-97	62 (23%)	137 (50%)	73 (27%)	125	(46%)
Germany	203	(4%)	50	55	0-93	33 (17%)	81 (41%)	84 (42%)	105	(55%)
Greece	67	(1%)	22	8	1-90	44 (66%)	12 (18%)	11 (16%)	37	(55%)
Italy	83	(2%)	38	39	0-94	20 (24%)	41 (50%)	21 (26%)	48	(59%)
Romania	33	(1%)	29	28	0-83	14 (42%)	17 (52%)	2 (6%)	17	(52%)
Sweden	558	(10%)	65	70	0-99	26 (5%)	165 (30%)	367 (66%)	281	(50%)
United Kingdom	3630	(66%)	49	48	0-104	443 (12%)	1,667 (47%)	1,469 (41%)	1953	(55%)
All countries	**5522**	**(100%)**	**50**	**52**	**0-104**	722 (13%)	2,460 (45%)	2,283 (42%)	**2912**	**(53%)**

5.2. Rates of severe *S. pyogenes* infection

Rates were calculated for all countries except Germany, France and Greece owing to incomplete participation among hospitals across these countries' catchment areas or within any definable catchment zone. Of the remaining eight countries, rates were calculated nationally for Cyprus, Czech Republic, Denmark, Finland and Sweden and for the following areas in other countries - Italy (Lombardia region), Romania (Bucharest and 7 other counties), UK (England, Wales, Northern Ireland, Channel Islands and Isle of Man). Of these eight countries, age-standardised rates were calculated and compared to crude rates. Standardisation had the biggest impact on Sweden in reducing its rate from 3.10 to 2.28 per 100,000 population (Figure 7).

Figure 7 **Crude and age-standardised annual rates of severe *S. pyogenes* infection reports, Europe 2003-2004**



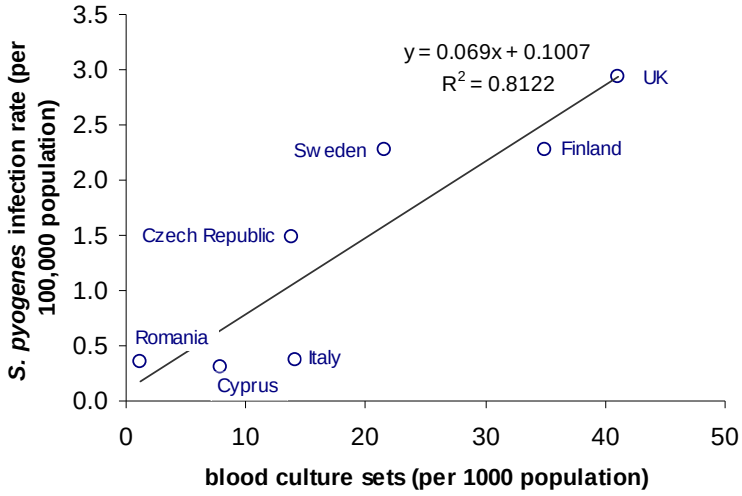
Age-standardised rates of severe *S. pyogenes* infection reports varied substantially (Figure 8). The overall rate for the eight countries was 2.37 per 100,000 population. A general north-south pattern could be discerned among participants, with the four most northern European countries having the highest rates of reports, 2.58/100,000 in combination. Within this group, rates in the UK (2.94) were significantly higher than

those for Finland or Sweden (2.28 for each; RR=0.78, 95% CI: 0.65-0.92) or Denmark (2.19; RR=0.74; 95% CI: 0.63-0.89). Among the two central European countries, the highest rate was reported for the Czech Republic (1.48), with rates for Romania (0.36) more similar to those observed in Italy (0.40) and Cyprus (0.30).

Figure 8 **Annual age-standardised rates of severe *S. pyogenes* infection by country, Europe 2003-04**

Age-adjusted rates of severe *S. pyogenes* infection were plotted against data supplied by hospitals participating in the European Antimicrobial Resistance Surveillance Scheme on rates of blood culture sets according to estimated catchment populations. Data were available for the following Strep-EURO/EARSS participants: Cyprus, Czech Republic, Finland, Italy, Romania, Sweden and the UK (Figure 9). A strong positive correlation could be seen between country-specific rates of infection and rates of blood cultures sets examined (Spearman's r = 0.92, p=0.003).

Figure 9 **Correlation between country-specific rates of blood culture sets taken in 2004 and rates of severe *S. pyogenes* infection in 2003-04**

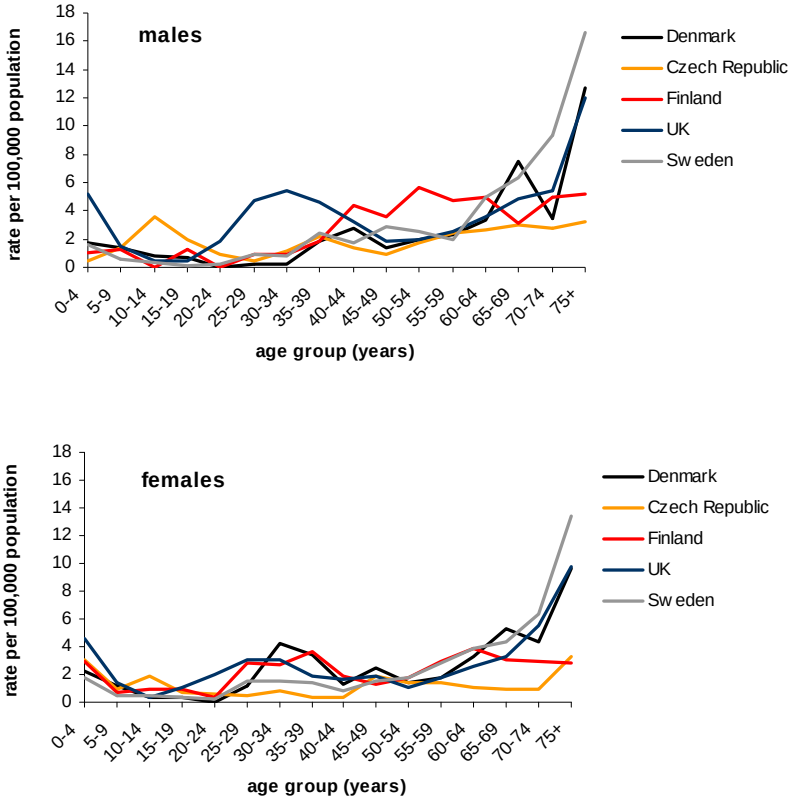


5.3. Age and sex-specific rates of infection

Age and sex-specific rates of severe *S. pyogenes* infection were calculated for the Czech Republic, Denmark, Finland, Sweden and the UK and sex-specific rates only for Cyprus, Italy and Romania owing to the small numbers of cases reported. In all countries bar Denmark, the rates of infection reports were higher in males than females (Figure 10), overall 3.03 vs 2.55 per 100,000 population (RR=1.19; 95% CI: 1.12-1.26).

Rates of infection were highest in the elderly, and to a much lesser extent in young children (0-4 years old; Figure 10). For Finland and the Czech Republic, the elevation in the elderly (75+ years) was much less pronounced than for the other countries (less than 6/100,000 for both sexes), whereas in Sweden the reverse was true, with rates of 16 and 13/100,000 in males and females respectively. A slight elevation in women of child-bearing age could also be discerned, most markedly in Denmark. Data from the UK were unusual in showing a prominent elevation in young males (25-44 years old).

Figure 10 **Annual age-specific rates of severe *S. pyogenes* infection, 2003-04**



5.4. Representativeness of cases with questionnaires returned

Of the 5522 severe *S. pyogenes* cases identified, questionnaires were returned for 79% (4334 cases). Comparison of variables available for cases with and without questionnaires returned identified those with questionnaires to be slightly older than cases without questionnaires (median age 53 vs 45; Kruskal-Wallis Test $\chi^2_{(1\ df)}=7.24$; $p<0.01$), although their sex distribution was similar ($\chi^2_{(1\ df)}=1.11$; $p=0.29$) as was peak season onset (January-March; ($\chi^2_{(1\ df)}=3.07$; $p=0.08$)). Analysis of UK cases linked to the national deaths registry identified a significantly higher proportion of cases with

questionnaires returned to have died from any cause within seven days (18%) compared to cases without questionnaires (12%; ($\chi^2_{(1\ df)}$=19.34; p<0.001)).

5.5. Clinical manifestations of *S. pyogenes* infection

Clinical information was available for 3894 (71%) of cases in total (Table 8). Of these, 19% (631) had a disseminated infection with no focus of infection reported. None of the cases from Romania and only 2 of 179 (1%) from Germany had a non-focal infection, whilst 10 to 26% of cases in other countries were reported as bacteraemic without focal symptoms. Of the focal infections, cellulitis was the most commonly reported overall (32%) and for the majority of countries, with the exception of the Czech Republic, France and Italy where necrotizing fasciitis was more often identified. Data on cellulitis were not collected from Sweden. In total, 308 cases of necrotizing fasciitis were identified, 8% of all cases. Necrotizing fasciitis was more common among male cases than female (9% vs 7%; $\chi^2_{(1\ df)}$=3.99; p=0.05), although not significantly so if injecting drug users (IDUs) are excluded ($\chi^2_{(1\ df)}$=2.45; p=0.12). Other presentations reported included septic arthritis (9%), puerperal sepsis (3%) and meningitis (2%). France reported relatively high numbers of puerperal sepsis cases, 9%, compared to other countries (5% or less).

Table 8 **Clinical presentations of severe cases of *S. pyogenes* infection, Europe 2003-04**

	cases with clinical information		bacteraemia with no focal symptoms		necrotizing fasciitis		cellulitis		septic arthritis		puerperal sepsis		meningitis		other *	
	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)
Cyprus	4	(100%)	1	(25%)	0	(0%)	1	(25%)	0	(0%)	0	(0%)	0	(0%)	2	(50%)
Czech Republic	136	(99%)	26	(19%)	28	(21%)	23	(17%)	12	(9%)	0	(0%)	4	(3%)	32	(24%)
Denmark	254	(91%)	51	(20%)	16	(6%)	67	(26%)	14	(6%)	12	(5%)	8	(3%)	92	(37%)
Finland	20	(8%)	2	(10%)	1	(5%)	3	(15%)	1	(5%)	1	(5%)	2	(10%	10	(50%)
France	272	(100%)	39	(14%)	48	(18%)	40	(15%)	26	(10%	25	(9%)	13	(5%)	67	(25%)
Germany	179	(88%)	2	(1%)	22	(12%)	26	(15%)	3	(2%)	2	(1%)	11	(6%)	97	(55%)
Greece	66	(99%)	14	(22%)	5	(8%)	25	(38%)	8	(12%	0	(0%)	3	(5%)	14	(22%)
Italy	72	(87%)	19	(26%)	14	(19%)	9	(13%)	2	(3%)	2	(3%)	0	(0%)	31	(43%)
Romania	32	(97%)	0	(0%)	4	(14%)	9	(31%)	1	(3%)	0	(0%)	1	(3%)	14	(48%)
Sweden	486	(87%)	NA		51	(11%)	NA		73	(16%	13	(3%)	0	(0%)	NA	
UK	2373	(65%)	477	(21%)	119	(5%)	833	(37%)	209	(9%)	52	(2%)	19	(1%)	1052	(47%)
All countries	**3894**	**(71%)**	**631**	**(19%)**	**308**	**(8%)**	**1036**	**(32%)**	**349**	**(9%)**	**107**	**(3%)**	**61**	**(2%)**	**1411**	**(43%)**
Death within 7 days	-		100	(20%)	80	(32%)	152	(17%)	28	(9%)	4	(4%)	12	(23%	275	(24%)

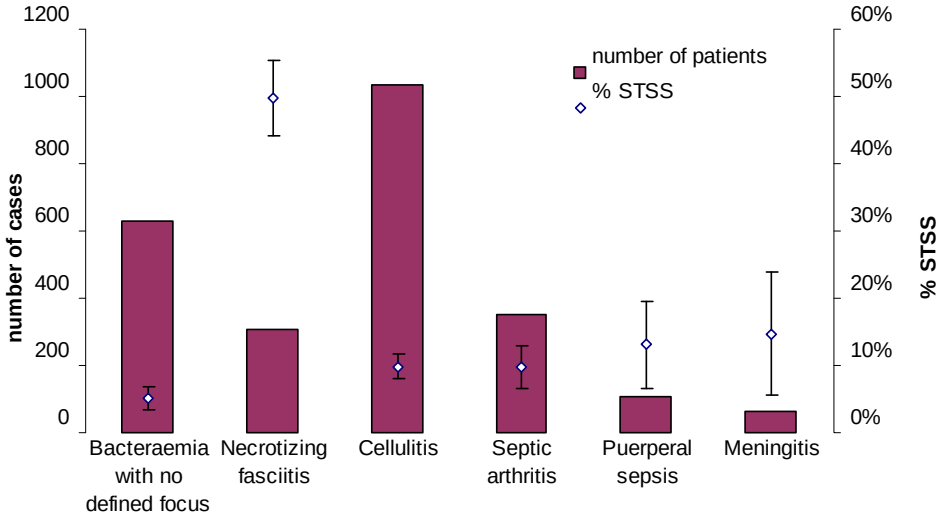
NA = not available.

* Excluding cases with necrotizing fasciitis, cellulitis, septic arthritis, puerperal sepsis or meningitis.

5.6. Development of STSS

Overall, 13% (493/3894) of cases developed STSS, rising to 50% among cases of necrotizing fasciitis. Between 10 and 15% of patients with other clinical presentations developed STSS, aside from cases with non-focal infections, only 5% of whom developed STSS (Figure 11).

Figure 11 **Development of STSS according to clinical presentation, Europe 2003-04**



Risk of STSS was highest among adults aged 16-60 years old (Table 9), 17% of whom developed STSS compared to 10% of paediatric or elderly cases. Across all age groups, necrotizing fasciitis patients had the highest likelihood of STSS, reaching 55% in those aged 16-60.

Table 9 **Risk of STSS according to clinical presentations and age, Europe 2003-04**

N=3733	**children** (<16y)	**adults** (16-60y)	**adults** (>60y)
	no. with STSS (%)	no. with STSS (%)	no. with STSS (%)
All cases	49 (10%)	277 (17%)	166 (10%)
Bacteraemia with no defined focus	1 (1%)	14 (6%)	17 (6%)
Necrotizing fasciitis	5 (29%)	100 (55%)	47 (44%)
Cellulitis	3 (3%)	54 (13%)	45 (9%)
Septic arthritis	4 (5%)	19 (12%)	11 (10%)
Puerperal sepsis	0 (0%)	14 (13%)	0 (0%)
Meningitis	2 (12%)	7 (24%)	0 (0%)

Multivariable analysis was undertaken to identify microbiological, demographic, clinical and risk factors potentially associated with development of STSS. Analyses were restricted to patients with sterile site isolates only to allow site of isolation to be examined as a possible predictor of STSS, given that the only patients with non-sterile site isolates included in the study were those with STSS. These analyses identified younger adults (16-60y; OR=3.0) and the elderly (>60y; OR=1.4) to be at higher risk of STSS than children, as were patients with necrotizing fasciitis (OR=4.4), those with post-surgical infections (OR=1.88) or patients infected with *emm*/M3 (OR=3.7) or *emm*/M1 (OR=3.0) compared to *emm*/R28. In contrast, injecting drug users (IDUs) were less likely to develop STSS (OR=0.2) than other patients, as were patients with cellulitis (OR=0.7) or those with non-focal infections (OR=0.4).

5.7. Clinical data from the UK

Within each country, considerable numbers of cases presented with conditions other than those detailed in Table 8, between 22 and 50% of cases per country. Further exploration of data from the UK identified pneumonia as being the most common of these other presentations, identified in 12% of patients (Table 10). Analysis of data from UK IDUs identified this group as being at particular risk of pneumonia (see 5.10 **Injecting drug users in the UK**).

Table 10 **Clinical presentations among cases of severe *S. pyogenes* infection, UK 2003-04**

	all cases[*]		**children** (< 16y)		**adults** (16-60y)		**adults** (> 60y)	
	no.	(%)	no.	(%)	no.	(%)	no.	(%)
Bacteraemia with no defined focus	558	(21%)	77	(24%)	228	(19%)	252	(24%)
Skin/soft tissue infection	1099	(42%)	85	(27%)	479	(39%)	531	(50%)
Cellulitis	881	(34%)	59	(19%)	362	(30%)	457	(43%)
Necrotizing fasciitis	136	(5%)	4	(1%)	87	(7%)	45	(4%)
Abscess	134	(5%)	6	(2%)	112	(9%)	16	(2%)
Erysipelas	24	(1%)	2	(1%)	9	(1%)	13	(1%)
Respiratory tract infection	434	(17%)	66	(21%)	187	(15%)	181	(17%)
Pneumonia	309	(12%)	27	(8%)	139	(11%)	143	(13%)
Other lower respiratory tract infection	62	(2%)	6	(2%)	26	(2%)	30	(3%)
Pharyngitis/tonsillitis	51	(2%)	26	(8%)	16	(1%)	9	(1%)
Ear infection	22	(1%)	8	(3%)	9	(1%)	5	(<1%)
Epiglottitis	17	(1%)	2	(1%)	10	(1%)	5	(<1%)
Sinusitis	6	(<1%)	4	(1%)	0	(0%)	2	(<1%)
Septic arthritis	220	(8%)	40	(13%)	114	(9%)	66	(6%)
Puerperal sepsis	58	(2%)	0	(0%)	58	(5%)	0	(0%)
Acute abdomen[†]	49	(2%)	5	(2%)	31	(3%)	12	(1%)
Cardiac infection[‡]	48	(2%)	2	(1%)	36	(3%)	10	(1%)
Meningitis	37	(1%)	18	(6%)	12	(1%)	7	(1%)
	2611	(100%)	318	(100%)	1225	(100%)	1064	(100%)

[*] Includes cases without age information.

[†] Includes 14 patients with peritonitis, 1 with appendicitis, 1 with pancreatitis.

[‡] 42 patients with endocarditis, 4 patients with pericarditis, 1 with myocarditis, 1 with myocarditis & pericarditis.

5.8. Factors predisposing severe *S. pyogenes* infection

Information on risk factors was available for 3178 (58%) of the cases. For 21% (683) of patients, no particular risk factors were identified, varying substantially between countries from 0% to 27% (Table 11).

Table 11 **Risk factors reported among cases of severe *S. pyogenes* infection by age, Europe 2003-04**

N=3176	**children** (<16y)	**adults** (16-60y)	**adults** (>60y)
	no. (%)	no. (%)	no. (%)
Skin lesion/wound	65 (18%)	284(22%)	332 (32%)
Immunocompromised	7 (11%)	43 (15%)	33 (10%)
Diabetes	0 (0%)	19 (7%)	47 (14%)
Injecting drug use	1 (2%)	59 (21%)	0 (0%)
Surgery	0 (0%)	3 (1%)	2 (1%)
Chickenpox	7 (11%)	2 (1%)	0 (0%)
Immunocompromised	30 (8%)	226 (16%)	272 (20%)
Injecting drug use	1 (<1%)	467 (32%)	3 (<1%)
Diabetes	3 (1%)	66 (5%)	192 (14%)
Chickenpox	73 (20%)	7 (1%)	2 (<1%)
Hospital-acquired infection	21 (5%)	117 (8%)	127 (9%)
Prior surgery	15 (71%)	73 (62%)	78 (61%)
No identified risk factors	144 (37%)	241 (17%)	297 (22%)
	388 (100%)	1440 (100%)	1348 (100%)

The most common single risk factor reported across all age groups was skin lesion, reported in 25% of cases overall (681), rising to 32% among the elderly (Tables 11, 12); 10% of these patients had diabetes (66) and 9% (60) were injecting drug users. Amongst children (<16 years old), chickenpox was the most common risk factor, reported in 20% (73) of cases overall. In total, 471 (15%) cases were IDUs, 93% (440) of these from the UK where 22% of cases were IDUs compared to 6% or less in other countries. Among young adults (16-60 years old), injecting drug use was the commonest risk factor, identified in 32% of cases.

Nearly half (45%; 21) of the cases from Greece were patients with chickenpox, a reflection of the largely paediatric setting of the surveillance in this country, all bar one of these 21 cases being children (Table 12). Other notable differences between countries included the proportion of patients with diabetes in the Czech Republic (22%) and Sweden (14%). This proportion remained high even among patients less than 75 years old, with 20% and 12% of Czech and Swedish cases respectively in this age group noted as having diabetes, compared to less than 6% in all other countries. Eight per cent of cases (265) were associated with healthcare interventions, although higher in the Czech Republic and France, 26% and 17% respectively, with post-surgical infections accounting for most of these.

Table 12 **Risk factors reported among cases of severe *S. pyogenes* infection by country, Europe 2003-04**

	cases with risk factor information		diabetes		IDU		chickenpox		immunocompromised		skin lesions		surgery		healthcare-associated infection		other *		none reported	
	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)
Cyprus	4	(100%)	0	(0%)	0	(0%)	0	(0%)	2	(50%)	1	(25%)	0	(0%)	0	(0%)	1	(25%)	1	(25%)
Czech Republic	72	(53%)	16	(22%)	2	(3%)	3	(4%)	23	(32%)	5	(7%)	11	(15%)	19	(26%)	23	(32%)	0	(0%)
Denmark	235	(85%)	13	(6%)	6	(3%)	3	(1%)	16	(7%)	42	(18%)	0	(0%)	26	(11%)	80	(34%)	61	(26%)
Finland	20	(8%)	1	(5%)	0	(0%)	1	(5%)	1	(5%)	10	(50%)	1	(5%)	2	(10%)	3	(15%)	3	(15%)
France	151	(56%)	10	(7%)	0	(0%)	5	(3%)	20	(13%)	38	(25%)	19	(13%)	25	(17%)	61	(40%)	0	(0%)
Germany	50	(25%)	0	(0%)	0	(0%)	3	(6%)	9	(18%)	23	(46%)	3	(6%)	6	(12%)	16	(32%)	0	(0%)
Greece †	47	(70%)	2	(4%)	3	(6%)	21	(45%)	7	(15%)	13	(28%)	4	(9%)	4	(9%)	3	(6%)	0	(0%)
Italy	48	(58%)	3	(6%)	3	(6%)	1	(2%)	9	(19%)	14	(29%)	2	(4%)	5	(10%)	10	(21%)	11	(23%)
Romania	26	(79%)	0	(0%)	1	(4%)	1	(4%)	3	(12%)	10	(38%)	1	(4%)	1	(4%)	5	(19%)	5	(19%)
Sweden	483	(87%)	67	(14%)	16	(3%)	NA		82	(17%)	NA		16	(3%)	23	(5%)	188	(39%)	128	(27%)
UK	2042	(56%)	150	(7%)	440	(22%)	44	(2%)	356	(17%)	525	(26%)	109	(5%)	154	(8%)	167	(8%)	474	(23%)
All countries	**3178**	**(58%)**	**262**	**(8%)**	**471**	**(15%)**	**82**	**(3%)**	**528**	**(17%)**	**681**	**(25%)**	**166**	**(5%)**	**265**	**(8%)**	**557**	**(18%)**	**683**	**(21%)**

* Excluding cases with any other risk factors as specified in the table.

† Cases primarily drawn from a children's hospital.

NA = not available.

5.9. Ethnicity of patients from the UK

Data collected on the UK cases included information on the ethnic group that patients belonged to. Ethnicity was recorded for 48% (1822) of patients in total (69% of patients with questionnaires). Of these 1822 cases, 95% (1727) were described as white, 3% (58) from the Indian subcontinent and 1% (21) black African or Caribbean. Rates of severe *S. pyogenes* disease observed were significantly higher among whites (3.29) than those of Indian subcontinent (2.46; RR=1.34; 95% CI: 1.02-1.74) or black African or Caribbean origin (1.79; RR= 1.91; 95% CI: 1.16-2.75).

5.10. Injecting drug users in the UK

5.10.1. Geographical and temporal distribution of cases

All countries within the UK reported cases of severe *S. pyogenes* infection in injecting drug users (IDUs) during 2003 and 2004, although IDUs constituted substantially higher proportions of all cases in England (21%) and Scotland (16%) than Wales (4%) or Northern Ireland (3%). Within England, cases in IDUs were most highly concentrated in the north of the country, especially within the Yorkshire and the Humber region where 44% of all cases were in IDUs in 2003-04, rising to nearly half (47%) of all cases in 2003 (38% in 2004). The North West and East Midlands also had high concentrations of IDU cases, 32% in 2003, falling to 25% and 20% respectively in 2004. The London region also reported relatively high numbers of IDU cases, 41% in 2003 falling to 20% in 2004. In contrast, the South West (17%), West Midlands (11%), North East (11%) and East of England (5%) all saw relatively fewer cases in IDUs over the two years. Spatial and temporal analysis of cases in IDUs identified 30% (138) to form part of one or more IDU clusters, being diagnosed within 10km and 30 days of another case in an IDU. A total of 87 clusters were identified, with a median and mean size of 5 cases (max 13).

5.10.2. Clinical presentations in IDUs

Of the 459 cases in IDUs, 96% (442) had positive blood cultures, higher than the proportion of non-IDUs (90%; $\chi^2_{(1\ df)}$=19.69; p<0.001). Skin and soft-tissue isolates were cultured with similar frequency in both groups, 15% of IDUs and 17% of non-IDUs. Clinical information was given for all cases except three of the IDUs and two of the non-IDUs. A similar proportion of both groups had a disseminated infection without obvious clinical focus, 22% of IDUs and 20% of non-IDUs. Of the focal sites for infection, skin and soft-tissue was the most common for both, although higher for non-IDUs (46%) than IDUs (37%). Abscesses were substantially more common in IDUs (18%) than non-IDUs (2%). Cardiac infections (endocarditis, myocarditis and pericarditis) were reported in 7% of IDUs, compared to 1% in other cases, with two-thirds (30/47) of all cardiac infections reported being in IDUs. Deep vein thrombosis was similarly more common in IDUs, 6% vs <1% in non-IDUs.

Respiratory tract infections were reported with similar frequency in IDUs and non-IDUs, although pneumonia was slightly more common in IDUs (14%) than other cases (11%), and twice as common in IDUs than non-IDUs of a similar age (15-44 years old, 7%; $\chi^2_{(1\ df)}$=9.51; p=0.002). Multivariable analysis of data from all UK cases indicated a three-fold increased likelihood of pneumonia in IDUs compared to non-IDUs (OR=3.00, 95% CI: 1.63 to 5.51, p<0.001). Other factors found to independently predict development of pneumonia were diagnosis in December (OR=2.04, 95% CI: 1.06 to 3.92, p=0.032) compared to a January baseline, and being aged 50-59 (OR=2.01, 95%CI=1.03 to 3.92, p=0.04) or 70-79 (OR=2.02, 95% CI: 1.09 to 3.77, p=0.026) compared to a 0-10 year old reference group. Patients infected with an *emm*/M83 strain (OR=0.38, 95% CI: 0.16 to 0.89, p=0.027) were also found to have a significantly lower risk of pneumonia than patients infected with other *emm*/M-types, with *emm*/M83 identified in 16% of IDUs with pneumonia compared to 23% of IDUs with other clinical presentations.

Comparison of clustered and non-clustered IDUs identified the latter to more commonly present with a respiratory tract infection, 18% vs 10% ($\chi^2_{(1\ df)}$=4.71; p=0.03), in particular pneumonia, diagnosed in 17% of non-clustered cases compared to 8% of clustered IDUs ($\chi^2_{(1\ df)}$=5.68; p=0.017).

5.10.3. Microbiological characteristics of IDU isolates

Corresponding isolates were received for 327 of the 459 IDUs (71%), and 1431 (77%) of non-IDUs. A total of 44 *emm*/M-types were present among the 327 IDU isolates, compared to 61 among the 1431 non-IDU isolates. Distribution of *emm*/M-types was markedly different between the two groups, most notably the high proportion of *emm*/M83 strains which constituted 22% of IDU infections and only 2% of non IDUs' ($\chi^2_{(1\ df)}$=186.07, p<0.001). Seventy per cent of all infections caused by *emm*/M83 were in IDUs. The next most common type identified in IDUs was *emm*/M87 (11% vs 10% in non-IDUs), followed by *emm*/M82 (9% vs 1%), *emm*/M43 (6% vs 1%) and *emm*/M33 (6% vs <1%). The following types were only identified in IDUs: *emm*/M88 and *emm*/M94 (2 cases each) and 1 case each of the following - *emm*/M27, *emm*/M92, *emm*/M102, *emm*/M25, *emm*st4986. The first and second most common types in non-IDUs, *emm*/M1 and *emm*/M3, were relatively uncommon in IDUs (3% and 2% of isolates respectively).

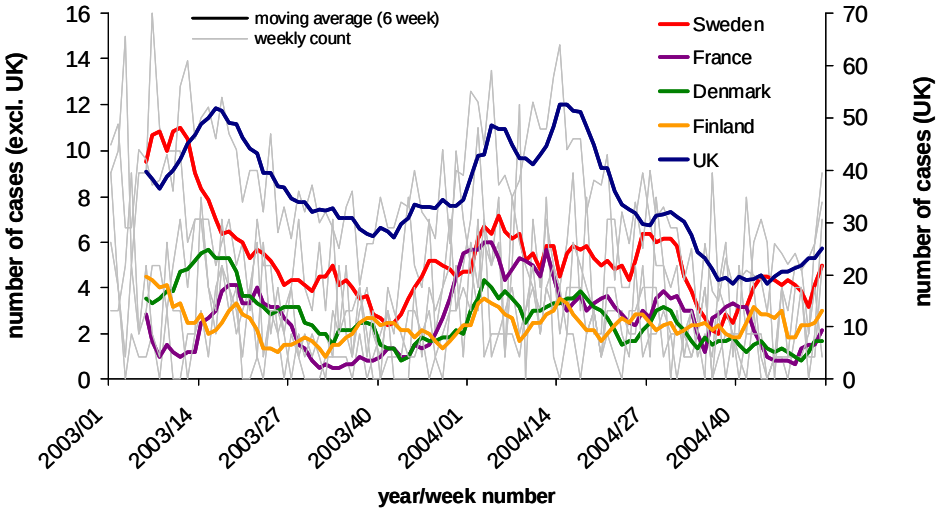
A greater diversity of *emm*/M-types was apparent among the 83 clustered IDUs with isolates available than the 244 non-clustered IDUs with isolates, with an average of three isolates per *emm* type for the clustered cases compared to six per type for the non-clustered cases. *emm*/M44 (5% vs 1%; $\chi^2_{(1\ df)}$=5.50, p=0.02), *emm*/M68 (4% vs 0%; $\chi^2_{(1\ df)}$=8.90, p=0.003), *emm*/M81 (8% vs 2%; $\chi^2_{(1\ df)}$=5.79, p=0.016), *emm*/M93 (2% vs 0%; $\chi^2_{(1\ df)}$=5.92, p=0.015) were over-represented among clusters, whereas *emm*/M89 (0% vs 7%; $\chi^2_{(1\ df)}$=5.72, p=0.017) was under-represented. Of the 87 individual IDU clusters, 67 (77%) had *emm*/M-typing results available from isolates from more than one member of the cluster. Of these 67 clusters, only 3 involved a single type: one cluster of three *emm*stNS1033 cases (two isolates); two further clusters of two *emm*/M83 cases.

5.11. Seasonal patterns of infection

Seasonal patterns of infection were examined for all countries who collected more than 250 cases over the two years: Denmark, Finland, France, Sweden and the UK. All countries observed higher numbers of infections in the winter and spring period, and marked low levels during the summer and autumn. March had the highest monthly excess of cases, 41% higher than the average monthly total. Seasonal trends in Finland

were less pronounced than for other countries, however. Striking similarities were observed in the timing of peaks and troughs between the five countries (Figure 12).

Figure 12 **Seasonal pattern of severe *S. pyogenes* infection by country**



5.12. Mortality following infection

5.12.1. Case fatality rates according to clinical presentation

Overall, 19% (643/3470) of patients died within seven days of diagnosis, with case fatality rates in the elderly substantially higher (32%) than children (9%) or younger adults (14%)(Table 13). Case fatality rates were highest among cases of necrotizing fasciitis, 32% overall (80/254) and rising to 42% in the elderly. Case fatality rates varied between countries, being highest for those reporting more cases of necrotizing fasciitis. Of the cases who developed STSS, 44% (184/415) died within seven days, with 31% (184/593) of all deaths being in patients with STSS. Of the small number of cases with meningitis, 23% (12/52) died, and 17% (152/895) of cases with cellulitis. Deaths in patients with septic arthritis (9%; 28/304) or puerperal sepsis (4%; 4/92) were less common. Although risk of death was highest among patients with necrotizing fasciitis, the highest number of deaths were in patients with cellulitis who constituted 27% of all

the deaths reported (152/553), or 25% if the 14 cases also diagnosed with necrotizing fasciitis are excluded (138/553).

Table 13 **Seven-day case fatality rates according to clinical presentations and age among cases of severe *S. pyogenes* infection, Europe 2003-04**

N=3733	**children** (<16y)	**adults** (16-60y)	**adults** (>60y)
	no. of deaths (%)	no. of deaths (%)	no. of deaths (%)
All cases*	34 (9%)	178 (14%)	341 (32%)
Bacteraemia with no defined focus	1 (1%)	25 (12%)	74 (33%)
Necrotizing fasciitis	0 (0%)	41 (28%)	39 (42%)
Cellulitis	3 (4%)	33 (9%)	116 (25%)
Septic arthritis	1 (1%)	13 (10%)	14 (14%)
Puerperal sepsis	0 (o%)	4 (4%)	0 (0%)
Meningitis	4 (29%)	6 (24%)	2 (15%)

* with clinical information reported

5.12.2. Case fatality rates according to risk factors

Seven-day case fatality rates varied substantially according to underlying risk factors. In children, younger adults and the elderly, those recorded as being immunocompromised for any reason had the highest fatality rates (Table 14), 17%, 20% and 32%. Injecting drug users had a considerably lower risk of death (6%) compared to other cases within the same age band. Of the cases without any predisposing factors, 9% and 10% of children and younger adults died with seven days, and 21% of the elderly cases.

Table 14 **Case fatality rates among cases of severe *S. pyogenes* infection by age and risk factor, Europe 2003-04**

N=2823	**children** (<16y)	**adults** (16-60y)	**adults** (>60y)
	no. of deaths (%)	no. of deaths (%)	no. of deaths (%)
All cases*	34 (10%)	159 (13%)	315 (26%)
Skin lesion/wound	2 (3%)	38 (15%)	91 (31%)
Immunocompromised	5 (17%)	41 (20%)	76 (32%)
Injecting drug use	0 (0%)	23 (6%)	0 (0%)
Diabetes	0 (0%)	10 (19%)	49 (28%)
Chickenpox	5 (8%)	0 (0%)	0 (0%)
Hospital-acquired infection	2 (11%)	12 (11%)	29 (25%)
No identified risk factors	11 (9%)	23 (10%)	57 (21%)

* with risk factor information reported

5.12.3. Independent predictors of poor survival

Multivariable analyses were undertaken to evaluate the potential predictive value of the following factors on patient death: demographic variables (age, sex), month of diagnosis, clinical presentations, patient risk factors, antimicrobial susceptibility and *emm* type. Several factors were found to independently predict risk of death, as outlined in Table 15, with the final model based on 1908 cases (the number of records with complete

information for all variables included in the model). Age was the strongest predictor, with risk of death increasing steeply with increasing age. Being immunocompromised was the only risk factor associated with risk of death, with these patients being 43% more likely to die within seven days than other patients. Interestingly, patients diagnosed in October had a considerably reduced likelihood of death, 86% less than patients in January (reference month). Of the main clinical presentations examined, several were found to carry a lower risk mortality compared to patients without these – non-focal infection, cellulitis, septic arthritis and puerperal sepsis. Development of STSS was the only presentation found to significantly predict death, with these patients having 4 times the likelihood of death than others (necrotizing fasciitis did not significantly predict death).

emm/M-types responsible for 30 or more infections were examined in the model. Three *emm* types were dropped by the model due to low numbers resulting in complete prediction of failure in the model (*emm*/M2, 73 and 118). Several *emm*/M-types were found to significantly predict death compared to the baseline comparator (*emm*/R28). The strongest association was for *emm*/M78 which was associated with over 3.5 times the risk of death, followed by *emm*/M5, *emm*/M3 and *emm*/M1. As a common type, the highest number of deaths were associated with *emm*/M1 infection (30%; 175/587).

Table 15 **Factors independently associated with mortality within seven days of severe *S. pyogenes* infection, 2003-04**

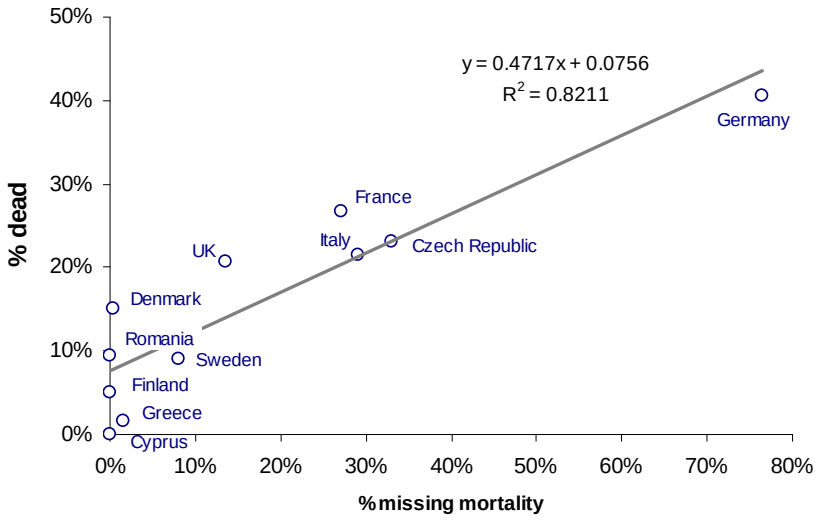
	no. cases	(% died)	adjusted OR	95% C.I.	p value
Patient characteristics					
Age group (years)					
< 1	82	(15%)	1	-	-
1-15	381	(9%)	2.19	(0.48 - 9.96)	0.312
16-30	368	(5%)	1.71	(0.35 - 8.22)	0.505
31-45	679	(13%)	3.38	(0.77 - 14.82)	0.106
46-60	484	(18%)	5.09	(1.16 - 22.32)	0.031
61-75	676	(25%)	8.04	(1.86 - 34.77)	0.005
over 75	798	(30%)	13.87	(3.22 - 59.75)	<0.001
Immunocompromise	479	(25%)	1.43	(1.05 - 1.96)	0.022
Month of diagnosis					
January	386	(23%)	1	-	-
February	347	(20%)	1.01	(0.60 - 1.68)	0.976
March	427	(20%)	0.91	(0.56 - 1.48)	0.711
April	413	(22%)	0.88	(0.54 - 1.43)	0.605
May	288	(18%)	0.93	(0.54 - 1.61)	0.793
June	257	(16%)	1.10	(0.61 - 1.99)	0.744
July	282	(17%)	0.88	(0.51 - 1.52)	0.640
August	197	(17%)	1.33	(0.71 - 2.48)	0.375
September	181	(12%)	0.61	(0.30 - 1.27)	0.188
October	190	(8%)	0.13	(0.04 - 0.44)	0.001
November	216	(16%)	0.86	(0.45 - 1.66)	0.660
December	286	(23%)	1.29	(0.75 - 2.25)	0.358
Clinical features					
Cellulitis	895	(17%)	0.50	(0.37 - 0.66)	<0.001
Septic arthritis	304	(9%)	0.36	(0.20 - 0.64)	0.001
Puerperal sepsis	92	(4%)	0.29	(0.08 - 1.04)	0.058[†]
Non-focal bacteraemia	506	(20%)	0.66	(0.46 - 0.94)	0.022
STSS	415	(44%)	4.03	(2.93 - 5.54)	<0.001
***emm*/M-type**[*]					
emm/M28	351	(14%)	1	-	-
emm/M11	31	(10%)	0.55	(0.07 - 4.49)	0.575
emm/M12	176	(17%)	0.99	(0.49 - 1.98)	0.974
emm/M18	43	(21%)	1.16	(0.41 - 3.24)	0.783
emm/M22	51	(12%)	0.58	(0.15 - 2.25)	0.431
emm/M3	305	(36%)	2.37	(1.43 - 3.94)	0.001
emm/M33	23	(4%)	0.16	(0.02 - 1.32)	0.089
emm/M4	146	(10%)	0.66	(0.29 - 1.51)	0.328
emm/M43	29	(21%)	1.87	(0.65 - 5.44)	0.248
emm/M5	71	(30%)	2.73	(1.30 - 5.72)	0.008
emm/M6	63	(17%)	1.08	(0.44 - 2.66)	0.865
emm/M75	45	(9%)	0.71	(0.15 - 3.39)	0.668
emm/M77	72	(21%)	2.12	(0.86 - 5.21)	0.103
emm/M78	29	(28%)	3.36	(1.16 - 9.76)	0.026
emm/M81	112	(10%)	0.62	(0.19 - 2.02)	0.428
emm/M82	34	(9%)	0.96	(0.26 - 3.60)	0.956
emm/M83	90	(8%)	0.77	(0.27 - 2.15)	0.615
emm/M87	187	(19%)	1.10	(0.60 - 2.04)	0.754
emm/M89	247	(13%)	0.69	(0.36 - 1.33)	0.268
emm/M1	596	(29%)	1.67	(1.05 - 2.68)	0.032
other	295	(11%)	0.48	(0.24 - 0.96)	0.037

* *emm*/M types with 30+ isolates; - reference group; [†] Likelihood Ratio Test p-value = 0.0318

5.12.4. Reporting of case fatality

Between countries, there was a general tendency towards higher case fatality rates being associated with less complete reporting of outcome (Figure 13; linear regression analysis R^2=0.82, p<0.001); cases from Germany had a particularly high mortality, 40%, although this information was only provided for 42 (21%) of their 179 cases. Conversely, cases from Finland and Sweden had a much lower mortality of 5% and 9%, with this information provided for 100% (in 2004) and 80% of their cases respectively.

Figure 13 **Correlation between completeness of mortality information and case fatality rate reported, Europe 2003-04**



Information on patient outcome for UK cases was available both through questionnaire reporting and through death registration records, with the latter identifying 16% (559/3422) of cases to have died within seven days of diagnosis (all cause mortality) compared to 21% (469/2237) of questionnaire responses ($\chi^2_{(1\ df)}$=19.51, p<0.001). Of the 469 deaths identified through the questionnaire, the cause was not known for 45 patients and 11 were noted as being due to other causes, leaving 413 (19%) deaths attributable to *S. pyogenes* infection.

For 2062 cases, information on seven day mortality was available through both the questionnaire and through linkage to the national deaths registry. Of these 2062

patients, the outcome at seven days was congruent between the two sources for 97% (2000/2065). Forty-six deaths identified through the study questionnaire were not substantiated by the deaths registry, although 20 of these 46 had died between 8 and 30 days after the initial positive culture. Of 1633 patients noted as being alive through the questionnaire, 19 were identified as having died within seven days through the deaths registry. The sensitivity of the questionnaire in identifying deaths within seven days was 95% (386/405) and the positive predictive value 89% (386/432).

5.13. *emm*/M-type distribution in the UK

Of the 3775 cases of severe *S. pyogenes* infection identified in 2003-04, isolates were available for 2493 (66%). Of these isolates, *emm* type was determinable for 2490 isolates using one of a number of techniques (see 4.3.2 **Collection and characterisation of isolates**). Comparison of information available for cases with and without accompanying isolates identified a higher likelihood of those with isolates to have subsequently died, as identified through the national deaths registry (11% vs 19%; $\chi^2_{(1\ df)}=32.93$, $p<0.001$).

A total of 75 different *emm*/M-types were identified, including eight provisional types (*emm*st11014, *emm*st1389.1, *emm*st4986, *emm*stD633, *emm*stG1750, *emm*stNS1033, st2037.1, stG6). The top 20 most common *emm*-types are given in Figure 14. Overall, *emm*/M1 was the most common type, identified in 18% of isolates, followed by *emm*/M3 (13%), *emm*/M87 (10%) and *emm*/M89 (8%).

Figure 14 **Distribution of *emm*/M-types causing severe *S. pyogenes* disease, UK 2003-04**

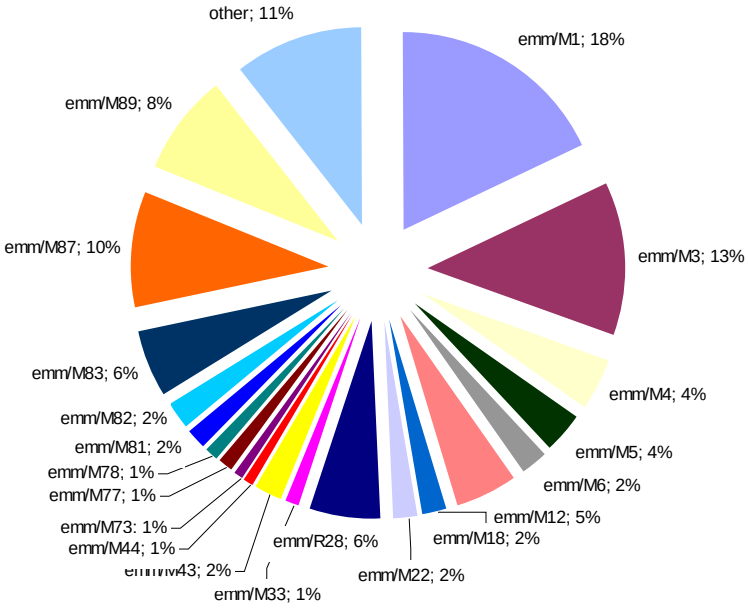


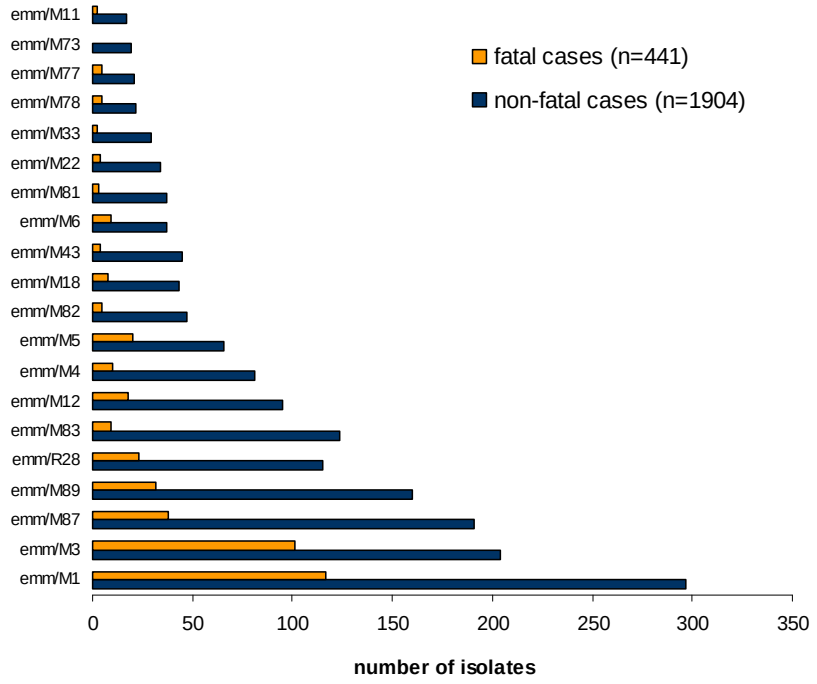
Table 16 **Association between *emm*/M-types* and clinical presentations in cases of severe *S. pyogenes* infection, UK 2003-04**

	***emm*/M1**		***emm*/M12**		***emm*/M3**		***emm*/M83**		***emm*/M87**		***emm*/M89**		***emm*/R28**	
	number	(%)	number	(%)	number	(%)	number	(%)	number	(%)	number	(%)	number	(%)
Bacteraemia with no defined focus	75	(18%)	14	(3%)	54	(13%)	38	(9%)	58	(14%)	35	(8%)	23	(5%)
Skin/soft tissue infection	168	(20%)	48	(6%)	99	(12%)	37	(4%)	84	(10%)	74	(9%)	53	(6%)
Cellulitis	125	(19%)	38	(6%)	75	(11%)	32	(5%)	69	(10%)	61	(9%)	48	(7%)
Necrotizing fasciitis	36	(34%)	6	(6%)	24	(22%)	4	(4%)	2	(2%)	9	(8%)	3	(3%)
Respiratory tract infection	68	(20%)	14	(4%)	50	(15%)	10	(3%)	26	(8%)	28	(8%)	24	(7%)
Pneumonia	50	(21%)	9	(4%)	35	(15%)	9	(4%)	18	(8%)	20	(8%)	15	(6%)
Septic arthritis	32	(20%)	11	(7%)	29	(18%)	9	(6%)	12	(7%)	12	(7%)	5	(3%)
Puerperal sepsis	6	(15%)	2	(5%)	6	(15%)	0	(0%)	9	(22%)	5	(12%)	7	(17%)
Meningitis	4	(15%)	3	(12%)	7	(27%)	0	(0%)	2	(8%)	1	(4%)	3	(12%)
All cases	369	(19%)	104	(5%)	250	(13%)	108	(5%)	204	(10%)	164	(8%)	121	(6%)

* *emm*/M-types identified in over 100 cases

Of the cases with non-focal bacteraemia, *emm*/M87 was overrepresented, accounting for 14% of these cases compared with 10% of cases overall ($\chi^2_{(1\ df)}$=3.79, p=0.05). No associations could be discerned between *emm*/M-type and skin and soft tissue infections as a whole, although among cases diagnosed with necrotizing fasciitis, *emm*/M1 (33%; $\chi^2_{(1\ df)}$=14.33, p<0.001) and *emm*/M3 (22%; $\chi^2_{(1\ df)}$=8.38, p=0.004) were overrepresented, whilst *emm*/M87 (2%; $\chi^2_{(1\ df)}$=8.19, p=0.004) was underrepresented (Table 16). Both *emm*/M87 and *emm*/M28 were associated with puerperal sepsis, each identified in 22% ($\chi^2_{(1\ df)}$=5.99, p=0.017) and 17% ($\chi^2_{(1\ df)}$=8.04, p=0.005) of cases respectively. No significant (single variable) associations were found between *emm*/M types and development of pneumonia (see 5.10.2 **Clinical presentations of IDUs** for results from multivariable analysis of factors associated with pneumonia). Among cases who died within seven days of infection (Figure 15), *emm*/M1 (27% of deaths) and *emm*/M3 (23% of deaths) were overrepresented. Conversely, *emm*/M83 (2% of deaths) was underrepresented among fatalities.

Figure 15 **Distribution of *emm*/M-types* causing severe *S. pyogenes* disease according to seven-day case fatality†, UK 2003-04**



* top 20 ranking *emm*/M-types; † within 7 days (patients may have died subsequently)

5.14. Susceptibility of UK isolates to antimicrobial agents

Antimicrobial susceptibility results were available for 2605 (69%) of UK cases. Of these, 4% of isolates were reported as erythromycin resistant, 15% tetracycline resistant and 2% clindamycin resistant. Of the erythromycin sensitive isolates, 1% were reported as clindamycin resistant, compared to 18% of the erythromycin resistant isolates ($\chi^2_{(1\ df)}$=65.63; p<0.001).

Resistance to erythromycin was also associated with tetracycline resistance, with 43% of erythromycin resistant isolates resistant to tetracycline compared to 14% of erythromycin sensitive isolates ($\chi^2_{(1\ df)}$=38.27; p<0.001).

Prevalence of erythromycin resistance fluctuated between 2 and 5% throughout the year, with no clear seasonal pattern. Resistance varied by age ($\chi^2_{(1\ df)}$=10.29; p=0.006), with cases in children having the lowest rates of erythromycin resistance (1%), compared to adults aged 16-60 (3%) or the elderly (5%). Infections associated with healthcare interventions also had a higher rate of erythromycin resistance (8% vs 4%; $\chi^2_{(1\ df)}$=3.64; p=0.06).

6. DISCUSSION

The aim of this study was to define the epidemiology of severe *S. pyogenes* infection in Europe in 2003-04, with a special emphasis on the UK. Analysis and interpretation of data collected would be of value to individual countries in assessing their burden of infection and evaluating the importance of different risk groups. Pooling of data across nations would further allow an analytical evaluation of important factors predicting patient outcome. The main findings and limitations of this work are discussed below.

6.1. Interpretation of findings from the study

6.1.1. Rates of infection across Europe

Estimation of the incidence of severe *S. pyogenes* infection during this project identified a general north-south gradient, from high to low (II). Remarkably similar age-standardised rates of reports were observed among the three Nordic participants – Finland, Denmark and Sweden – between 2.2 and 2.3 per 100,000 population. Rates in the UK were higher still, 2.9 per 100,000 or 3.3 if unadjusted, closer to the rates observed in the USA and Australia during this period[2;200;201]. The large number of cases in injecting drug users (IDUs) in the UK, 21% of all the UK's cases compared to 6% or less in other countries, are likely to have elevated this rate.

In contrast, the rates of reports observed in the more central and southern countries – the Czech Republic, Romania and Italy – were substantially lower, 0.4 to 1.5 per 100,000 population. Although these lower rates might reflect a true lower incidence of infection, they are more conceivably explained by a poorer uptake of microbiological investigative methods within these countries, further substantiated by the strong correlation with rates of blood culture sets taken among participants in EARSS[107]. Failure to report diagnosed cases could also account for the lower rates observed in some countries; evidence to support this assertion would be difficult to find.

6.1.2. Predictors of death

One in five of cases identified during the study was reported to have died within seven days of infection (II). This figure, like most estimates from the study, is highly influenced by the large body of data from the UK. As such, it should be interpreted with particular caution given that survival data from the UK pooled with the other countries were obtained from the study questionnaire, rather than from any external and objective source. The outcomes of cases of whose fate was unknown are probably less likely to have died, resulting in an overinflation of this estimate to some degree. This is supported by the (inverse) correlation between completion of these data and case fatality rate between countries, along with the subsequent analysis of UK deaths registry data which identified a lower proportion of patients to have died (16%) than identified from the questionnaire alone (21%)(IV). However, this overall case fatality rate does lie within the range observed in other studies[2-8].

Several factors were identified as being associated with increased risk of death. Many have been identified by other studies, such as age, immune status, development of STSS and *emm*/M-type[1;4;11;117]. Despite its reputation, necrotizing fasciitis was not itself associated with increased risk of death, the high mortality in these patients being accounted for by the higher frequency of STSS, also found elsewhere[6]. Analysis of survival time in UK patients diagnosed with STSS illustrated the rapid deterioration of these patients, just over a quarter of whom died within a day of culture positive specimen collection. Whether STSS should be considered as an event independent of death is possibly questionable, as its constituent clinical markers effectively denote the progressive shutdown of major organ systems, along with DIC and development of an erythematous rash, and as such would seem to signal the start of the process of death.

Several studies have found pneumonia to be associated with increased risk of death, and whilst this presentation was not included in the European questionnaire(II), its inclusion in the UK questionnaire supports this finding(I). Our study also found two serotypes - *emm*/M78 and *emm*/M5 – to be independently associated with an even higher risk of death than *emm*/M3 and *emm*/M1, a finding which was potentially only discoverable with a sample size as large as this given the relative infrequency of these types as a cause of invasive disease. One further association was found which has not previously been documented, that mortality changes in parallel with season, with the highest case fatality

rates in peak season and the lowest in low season. The risk of death in patients infected in October was nearly 90% less than in patients diagnosed in January.

Whilst the findings above are of importance in identifying patients at higher risk of death, from a public health perspective, it's essential to also evaluate factors associated with high numbers of deaths (mortality, rather than case fatality). Whilst development of STSS was associated with a high risk of death, less than a third of deaths were in patients with STSS. Although often overlooked given its frequency and usually uncomplicated outcome, more deaths were associated with cellulitis than any other clinical syndrome.

6.1.3. Predictors of STSS

Analysis of factors associated with STSS amongst the European cases supported previously identified associations, namely heightened risk in young adults compared with children or the elderly, patients with necrotizing fasciitis and patients infected with *emm*/M3[6;117;119]. Injecting drug users were found to have a lower risk of STSS than other patients, a finding not reported elsewhere, as were patients with malignancies. Further analyses were undertaken with the more detailed UK data(I), which found alcoholism to be associated with increased risk of STSS, also found by others[4;7]. However, we found that patients recorded as having used non-steroidal anti-inflammatory drugs had three times the likelihood of developing STSS, despite adjustment for whether the patients had necrotizing fasciitis, itself strongly associated with STSS. As no data were collected specifying the timing, dose, indications for use or which agent was taken, one cannot infer a causal link between use of non-steroidal anti-inflammatory drugs and development of STSS from our findings. A confounding factor, such as delay in receiving appropriate treatment, not adjusted for in our analysis could explain the finding. It is also possible that patients who took non-steroidal anti-inflammatory drugs had early signs indicating a more severe infection, such as extreme pain. Nonetheless this remains an interesting and potentially important observation in a controversial area where evidence supporting either immunological impairment or augmentation due to non-steroidal anti-inflammatory drugs remains unresolved at present[144].

6.1.4. Seasonal patterns of infection

The similarities in the seasonal patterns of infection in different countries observed in this study are among the most striking of the results(II). The near simultaneous changes are difficult to explain as they point to an influence whose timing of effect would be very similar between countries. A climatic explanation would seem plausible, for example a change in humidity affecting mucosal defence, which could in turn also explain the east-west axis of low to high incidence in Canada, given the relatively dry and arid conditions in the west. Daily photoperiod is known to influence immunological defence, given that both vitamin D and melatonin production are affected by this cycle[146;202]. Interestingly, serum levels of vitamin D (25(OH)D3) are known to be lower in individuals with darkly pigmented skin[203], which could offer an explanation for the increased incidence reported in various non-white ethnic groups, although not found in the UK. Although the evidence for the importance of photoperiod on the winter excess of deaths is fairly compelling, and in particular deaths associated with influenza, the pattern of incidence for *S. pyogenes* infections does not follow a mid-summer low mid-winter high cycle[146;165]. Seasonal changes in behavioural patterns, either as a result of external temperature affecting indoor gathering versus outdoor pursuits or dictated by the academic calendar are likely to influence the chains of transmission. The long summer holidays may disrupt transmission, in particular among children, an important reservoir for many infectious diseases, although one might again expect the summer months to see the lowest incidence in this case. That the risk of death also changed with the season in parallel with the incidence of infection, is a novel and fascinating finding, which would fit with an immunological theory. Clearly some residual and unaccounted for factor influencing immunological function and connected to time of year is exerting an important effect.

6.1.5. Factors predisposing to infection

Analysis of factors potentially predisposing to severe *S. pyogenes* infection found that that a quarter to a fifth of cases in most countries had no particular factors predisposing to severe infection, based on responses made in the study questionnaire(II). A certain degree of caution should be exercised in interpreting these results as it was left to the discretion of the reporting clinicians (or country co-ordinators in further cleaning data) as to what might be considered a risk factor beyond the standard options given on the study questionnaire. In some countries, for example Sweden, coronary heart disease was

included within this list, a condition of sufficient commonality in the elderly that this could have some influence on the estimation of numbers of patients without risk factors. Of the patients without apparent risk factors, there may of course have been events or exposures which predisposed to infection which would not have been readily considered, such as presence of an intrauterine device[155]. Furthermore, it would be easy to overly infer that any risk factors which were mentioned were of relevance to the development of infection in any given patient. Many accepted 'risk factors' have no epidemiological evidence as their basis.

This limitation notwithstanding, some interesting findings emerged, the most striking of which was the high proportion of IDUs among UK cases (22%) compared to any other country (≤ 6%), discussed further below. Two countries reported substantially higher proportions of their cases being healthcare intervention related, the Czech Republic (26%) and France (17%), most other countries reporting approximately 10% of cases as being healthcare associated. Several maternity clusters occurred in France during this period, probably accounting for their figure, although it remains unclear why the proportion in the Czech Republic should be so high. A further notable difference between countries lies in the high proportion of cases from the Czech Republic (22%), and to a lesser extent Sweden (14%), having diabetes. The prevalence of diabetes in the general population is known to be high in the Czech Republic, possibly explaining their figure, although it remains unclear why the Swedish cases should be so different[204].

6.1.6. Upsurge of cases in UK drug injectors

Analysis of the wealth of information collected on cases of severe *S. pyogenes* infection in UK injectors indicates that a distinct epidemic occurred in this group in the UK(III), peaking in 2003[205]. Serotypes involved in causing disease in injectors were markedly different to those in non-injectors, with several serotypes only seen in injectors. These cases are clinically interesting – 14% of cases presented with pneumonia, indicating that non-injecting routes of infection were important in fuelling this epidemic. Multivariable analysis of data from all cases indicated a three-fold increased likelihood of pneumonia in IDUs compared to non-IDUs when adjusting for age, season and *emm*/M-type. Although single variable analysis suggested that IDUs were less likely to die than other cases, multivariable analysis failed to find any difference between IDUs and others, suggesting

that their better outcome is explained by the reduced likelihood of developing STSS and by the serotypes involved in their infection. That IDUs were less likely to develop STSS may relate to a greater natural immunity in IDUs through repeated infection with *S. pyogenes* over time, rendering IDUs more immunologically primed to respond to such infections and less susceptible to the common strains infecting non-IDUs.

The IDU cases seemed to be divided into two broad categories: clustered cases, infected with a diverse range of serotypes, presenting with range of clinical manifestations and more likely to be admitted from an institution; non-clustered cases, presenting with a range of infections but with more lower respiratory tract infection than clustered cases and infected with a narrower range of serotypes. These findings suggest that the clusters may be occurring in more marginalised injectors as a result of injecting behaviour, whereas the non-clustered cases are possibly infected through respiratory transmission.

The drivers behind this increase in injectors are not understood, although it is of interest to note that a similar increase was seen in Barcelona in 2003[206]. Increases in cocaine injection were considered to have led to an upsurge of severe *S. pyogenes* infection seen in IDUs in Philadelphia between 1979-89[207], injection of cocaine facilitating tissue necrosis through localised vasoconstriction. This could explain the rise seen in the UK, with anecdotal evidence indicating the adoption of a new marketing strategy by UK drug dealers during the 2000s to increase demand for crack-cocaine – provision of free crack 'rocks' with heroin purchases, allowing the two drugs to be dissolved and injected together ("speedballing"). An increase in cocaine injecting, would also explain the rise in outbreaks of anaerobic infections seen in the UK in the early 2000s[208-210].

6.1.7. Potential impact of vaccine candidates

The collection of around 2500 isolates involved in severe *S. pyogenes* infection in the UK permits an evaluation of the potential impact of multivalent vaccine candidates, in particular the two currently under trial. Assuming 100% efficacy, the hexavalent vaccine candidate could have potentially prevented 36% of the cases identified in 2003-04 and 56% of the associated deaths occurring within seven days[184]. In contrast, the 26-valent vaccine could have prevented 67% of cases (and 80% of deaths)[185], lower than recent estimates for Canada (72%), the USA (79%), Japan (82%) or Mexico (86%)

[1;94;98;211] but above the 22% recently reported in Hawaii[212]. Setting aside efficacy, any estimates of impact may be undermined by the potential for serotype replacement to occur subsequent to initiation of an immunisation strategy[213], which given the current experience with pneumococcal vaccination and suggested in at least one GAS carriage study[214], may well represent a real threat[215]. A range of different *emm*/M-types are involved in invasive disease, 75 in this analysis in the UK, outlining the pathogenic potential of less common types. Furthermore, this species is clearly still evolving, with novel types continuing to emerge, potentially further undermining any vaccine targeting the M-protein.

6.2. Methodological considerations

As with any scientific endeavour, a number of limitations are inherent to this work and as such certain considerations should be held in interpreting the data. The interpretation of the results discussed in 6.1 **Interpretation of findings from the study** are made taking these into account, and in turn these are reflected in 6.3 **Future considerations**. The main limitations and caveats are outlined below.

6.2.1. Design limitations

The main limitation of a surveillance study is the inability to make robust comparisons between cases and an external or control group of individuals. Although it is possible to compare the characteristics of cases to normative data collected at the population level as undertaken in some studies, this would be limited to factors for which normative data exist. Furthermore, as such data are collected using entirely different methodology, there exists the potential for making an invalid comparison.

Given these limitations, it is not possible to make any inferences on the importance of specific risks as one cannot measure the strength of association for which a control group is required. As some of the risk factors for severe *S. pyogenes* infection are reasonably common, such as cardiovascular disease, comparison of the frequency of this condition in cases has to be compared to a control group if it's to have any meaning. Nonetheless, the descriptive epidemiology of possible predisposing factors provided by surveillance studies is still of value in directing further research as well directing public health activity where clear excesses arise in particular subgroups.

6.2.2. Surveillance methods

One of the most important limitations of this work are the methodological differences between countries in collection of cases(II). This prevented estimation of rates of infection for three of the eleven participating countries, primarily owing to a lack of participation by hospitals in these countries. Under such circumstances, further concerns arise that biases may be introduced in the cases which are reported. Cases reported may be ones that were of particular interest owing to some novel feature, responded poorly to treatment or were particularly severe. Some evidence for the latter can be found from the Czech Republic, France, and Italy, all of whom reported one in five of their cases as having necrotizing fasciitis, suggestive of a possible bias either in the application of microbiological investigation or in the reporting of more severe cases.

Although less problematic than the above, two countries did not adopt the standardised surveillance questionnaire agreed by the project owing to ongoing surveillance activity. For one of these, Denmark, this would probably have had little effect as their questionnaire covered the items included in the Strep-EURO questionnaire. However for Sweden, their questionnaire did not capture the same range of clinical presentations. As such, no estimates for cellulitis could be made, nor for non-focal bacteraemia. As the second largest contributor to the programme, this represents a missed opportunity.

6.2.3. Participation rate

Inherent to questionnaire-based research is the potential for biased completion of questionnaires. Comparison of data available for European cases with and without questionnaires did not point to any differences in a number of factors(II), although cases with questionnaires were slightly older than others (median age 53 vs 45). However, a number of countries only identified cases through questionnaire completion or other active means (isolate referral), and as such comparative data do not exist for them. Furthermore, the factors that one would most like to have available for comparison, namely clinical presentations, risk factors and mortality, are the very ones usually not available for comparison. Within the UK, linkage of records for all cases to the national register of deaths provided an opportunity to examine a potential bias in questionnaire return or isolate referral(IV), and indeed found that cases for whom questionnaires were

completed or isolates submitted had a higher mortality than those without questionnaires or isolates. Although the same calculations cannot be made for other countries, it is likely that there could be biases present within their data collection, further supported by the observed correlation between case fatality rates and completion of outcome information and the varying severity of clinical presentations between countries.

6.2.4. Data collected

Studies on the scale of the one undertaken here will necessarily be limited in the number of data items collected. The choice of items included on a study questionnaire or microbiological analyses undertaken fundamentally shapes our ability to draw novel conclusions from any research – what is not looked for cannot be found. Multinational research projects are potentially even more prone to limiting themselves as to what they look for in that one person's hypothesis or experience is unlikely to convince a multitude of collaborators.

Although the study questionnaire devised for this project struck a reasonable balance between comprehensiveness and brevity, some key items ought to have been included. The clinical presentations listed should have included pneumonia and acute abdominal presentations given their frequency. Capture of data on treatment strategies would have also been valuable in assessing different approaches in different countries, in particular in the use of IVIG.

Owing to financial constraints, use of subtyping techniques in analysing isolates captured was rather limited, as was the identification of virulence markers, such as *spe* genes. The latter represents a missed opportunity as analysing the association between these and different clinical presentations and outcomes would have been of considerable value. However, all isolates have been archived, and as such these analyses could be undertaken in the future.

6.3. Future considerations

1. Continuation of surveillance of severe *S. pyogenes* infection to monitor changes in rates of infection, risk factors, clinical presentations, *emm*/M-type distribution and antimicrobial susceptibility patterns remains essential. Results from this study re-emphasise the dynamic nature of these infections and the need for constant vigilance to detect new and emerging patterns of disease;
2. Development of algorithms to routinely assess case fatality rates and absolute mortality to detect exceedances above those seasonally expected should be considered;
3. Comparison of seasonal patterns of infection from countries in different continents, along with correlations to environmental and social parameters, could yield some interesting insights into this interesting phenomenon;
4. A case-control study should be undertaken to evaluate the role of non-steroidal anti-inflammatory drugs as a potential risk factor for development of STSS;
5. An evaluation of the long term sequelae of severe *S. pyogenes* infection should be considered as a means of evaluating the burden of infection;
6. A detailed investigation of cases of severe *S. pyogenes* infection with no apparent risk factor or portal of entry should be undertaken to generate hypotheses on novel factors to be tested in analytical studies;
7. Guidelines should be developed on the investigation and management of clusters of severe *S. pyogenes* infection occurring in maternity settings;
8. The utility of primary care surveillance systems in forecasting epidemic rises in severe *S. pyogenes* infections should be assessed.

7. SUMMARY AND CONCLUSIONS

7.1. Key findings from the study

In accordance with the original study objectives, the following findings were made.

To measure and compare the overall and disease-specific burden of S. pyogenes *disease in eleven countries across Europe*

- In northern European countries, between 2 and 3 individuals per 100,000 population succumb to a severe *Streptococcus pyogenes* infection per year;
- Rates of severe *S. pyogenes* infection in central and southern European countries were lower, a probable reflection of the poorer application of microbiological diagnostic techniques in hospitals;
- Seasonal patterns of infection were very similar between countries, with near simultaneous timing in certain up- and downswings.

To identify and compare key risk groups in each country to potentially identify targets for public health intervention

- Males were at higher risk of infection than females, as were the elderly;
- The age distribution of patients in Finland was unusual in having low rates in the elderly;
- IDUs constituted a substantially higher proportion of cases in the UK than any other country, 40% of cases in adults aged 16-60y.

To identify factors associated with development of STSS as a means of directing future basic research into disease pathogenesis

- Development of STSS was highest in young adults, patients with necrotizing fasciitis, patients with post-surgical infections and patients infected with *emm*/M3 and *emm*/M1;

- Patients with cellulitis, patients with non-focal infections and injecting drug users were at lowest risk of STSS;
- Analysis of UK data found use of NSAIDs to be significantly associated with development of STSS.

To undertake an in-depth analysis of cases occurring in UK injecting drug users according to emm *types, clustering, clinical presentations and outcome to better understand the possible modes of transmission and burden of disease in this group*

- Clinical presentations in UK injectors were broadly similar to non-injectors, although drug injectors were more likely to present with pneumonia than other cases of the same age;
- *emm*/M-types in UK injectors were distinct from other cases arising during the same period, with a strong predominance of *emm*/M83;
- Temporal, spatial and microbiological analysis of data on cases in injectors suggests a distinct epidemic of infection occurred in the UK, peaking in 2003.

To better understand clinical, demographic, microbiological and other possible predictors of mortality

- Elderly patients, those with compromised immune systems, those who developed STSS and those infected with an *emm*/M78, *emm*/M5, *emm*/M3 and *emm*/M1 were most likely to die as a result of their infection;
- Non-focal infection, cellulitis, septic arthritis and puerperal sepsis were associated with low risk of death, as were infections occurring during the lowest season, October.

Analysis of data gathered in the eleven countries participating in the Strep-EURO programme has yielded invaluable new information on the epidemiology of severe *Streptococcus pyogenes* infections in Europe during the 2000s. The project succeeded in establishing the first European surveillance network for severe *S. pyogenes* infections,

through which over 5000 cases were identified over the two years, with a wealth of clinical and microbiological information accompanying the majority.

Analysis of project data has brought some new insights into risk factors for severe *S. pyogenes* infection, especially the importance of injecting drug use in the UK, with infections in this group fundamentally reshaping the epidemiology of these infections in the UK. Several novel findings arose through this work, namely the associations between NSAID and STSS, the high degree of congruence in seasonal patterns between countries, and the seasonal changes in case fatality rates. Analysis of data to identify household clusters in the UK has doubled the number of household clusters identified worldwide as part of a coherent surveillance activity. This has in turn allowed an evaluation of risk to contacts, forming the basis of guidance on the management of cases of *S. pyogenes* infection arising in the community.

Surveillance of invasive infections caused by *S. pyogenes* is of considerable public health importance as a means of identifying long and short-term trends in incidence, allowing the need for, or impact of, public health measures to be evaluated. By their very nature, *S. pyogenes* infections are dynamic, with changes in epidemiological patterns occurring frequently. Monitoring the prevalence of circulating *emm*/M-types provides an early warning of likely increases in incidence of severe infection, as well as providing a means to evaluate the likely impact of multivalent vaccines currently under development.

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10. ANNEXES

10.1. UK Strep-EURO questionnaire

ENHANCED SURVEILLANCE OF SEVERE GROUP A STREPTOCOCCAL INFECTION

RSIL ref no: 0…./……/SA **Name of laboratory**……

Your lab/hosp no…… **Isolate referred from (hospital & clinician name)**……

Address……

IN STRICT CONFIDENCE

☑ **Please tick boxes or write in the space(s) provided** (see notes overleaf)

i) PATIENT DETAILS

·patient's initials ☐ SOUNDEX ☐

·date of birth *(dd/mm/yyyy)* ☐

or age ☐ years/months/days *(please delete)* not known ☐ 999

·male ☐ 1 female ☐ 2 not known ☐ 9

·date of hospital admission *(dd/mm/yyyy)* ☐/☐/☐

·*Ethnicity:* white ☐ 1 black Caribbean ☐ 2 black African ☐ 3

Indian/Pakistani/Bangladeshi ☐ 4 mixed background ☐ 5

other *(please specify)* ☐ not known ☐ 9

ii) ISOLATE DETAILS

·Date of specimen *(dd/mm/yyyy* ☐/☐/☐

·*Isolated from:* blood ☐ 1 joint ☐ 2 wound ☐ 3 CSF ☐ 4

other *(please specify)* ☐

·Other relevant pathogens associated with this illness/episode?

yes ☐ 1 *(please specify)* ☐

no ☐ 2 not known ☐ 9

iii) CLINICAL DETAILS

·*Clinical presentation*: bacteraemia ☐ septic arthritis ☐

toxic shock-like syndrome ☐ cellulitis ☐ pneumonia ☐

necrotising fasciitis ☐ meningitis ☐ puerperal sepsis ☐

erysipelas ☐ other *(please specify)* ☐

·*Degree of severity:* hypotensive shock ☐ renal impairment ☐

DIC ☐ liver abnormality ☐ respiratory distress ☐

erythematous rash ☐ soft-tissue necrosis ☐

none of these ☐ not known ☐

·*Clinical management:*

admitted to I.T.U.? YES/NO/not known *(please delete)* If YES, no. days spent in ITU *(of the 7 days following GAS isolation)* ☐ days

surgical intervention? YES/NO/not known *(please delete)* *(please specify procedures)* ☐

·*Outcome* (at one week after GAS isolation) not known ☐ 9 alive ☐ 0

died – *GAS infection was main/underlying cause of death* ☐ 1

– *GAS infection contributed to death (not main cause)* ☐ 2

– *GAS did not contribute to death* ☐ 3 – *cause unknown* ☐ 4

iv) EPIDEMIOLOGICAL INFORMATION

·*Risk factors*: steroid use ☐ diabetes ☐ injecting drug user ☐

varicella ☐ non-steroidal anti-inflammatory drugs ☐

alcoholism ☐ malignancy ☐ skin lesion/wound ☐ If YES:

trauma ☐ 1 *insect bite* ☐ 2 *surgery* ☐ 3 *(please specify)* ☐

recent childbirth *(last 4 weeks)* ☐ If YES: date: ☐

vaginal deliver/caesarean section *(please delete)*

other risk factor/s including immunosupression or previous GAS disease (within last 14 days) *(please give details)* ☐

no identified risk factors ☐ information not known ☐

·*Other epidemiological information*

Occupation of patient: ☐

Recent overseas travel *(last 2 weeks)*? YES/NO/not known *(please delete)*

If YES, which country/ies? ☐

Was the patient admitted from an institution? YES/NO/not known

If YES, what type? ☐

Was this infection hospital acquired? YES/NO/not known *(please delete)*

Was this case related/contact of other case(s) of GAS disease? YES/NO/not known *(please delete)* If YES, please provide details -

relationship to *this* case: ☐

date of onset/specimen of related case: ☐/☐/☐

clinical presentation of related case: ☐

☐

ADDITIONAL INFORMATION

Please provide any additional information of interest

☐

☐

☐

--- thank you for completing this questionnaire ---

COMPLETED BY:……

TELEPHONE NO.: ……

TODAY'S DATE: ……/……/……

European Commission DG RTD QLK2.CT.2002.01398

Health Protection Agency use only

strep-EURO ID **GB** ☐ received ☐

ENHANCED SURVEILLANCE OF SEVERE GROUP A STREPTOCOCCAL INFECTION

strep-EURO - is a three year European Commission Framework Five programme for severe group A streptococcal (GAS) disease in Europe, launched on 1st September 2002. Its aim is to enhance our understanding of the epidemiology of GAS invasive disease in Europe. In fulfilment of the aims of the programme, nine countries are undertaking enhanced surveillance and isolate collection for all cases of severe GAS disease occurring between 1st January 2003 and 31st December 2004. It is hoped that this will provide valuable data to inform future treatment and vaccine strategies for severe GAS disease. The participating countries are Sweden, Germany, Finland, United Kingdom, Italy, Greece, Denmark, Czech Republic, Cyprus and Romania.

Public Health Laboratory Service. Enhanced surveillance of invasive group A streptococcal infections. *Commun Dis Report CDR Wkly [serial online] 2002* [cited 19 December 2002]; ***12*** (51).

Guidance on the completion of the form - please complete one reporting form for *each case* diagnosed in your laboratory between 1st January 2003 and 31st December 2004, meeting the following case definition of severe group A streptococcal disease:

> **Isolation of a group A streptococcus (*Streptococcus pyogenes*) from a site that is normally sterile: blood; cerebrospinal fluid; joint aspirates; pericardial/peritoneal/pleural fluids; deep tissue or abscess at operation or necropsy; bone.**

Please complete as much of this form as possible and return in the pre-paid envelope to: **Dr A Efstratiou, Health Protection Agency, Respiratory and Systemic Infection Laboratory, Specialist and Reference Microbiology Services Division, 61 Colindale Avenue, London NW9 5HT**. All information supplied will be treated as confidential; under no circumstances will individual case details be passed on to a third party. The following definitions will help with completion of the sections overleaf.

Section i) & ii)

- *SOUNDEX* – coding system for anonymisation of patient's surname (e.g. L265). Please supply if known.
- *date of hospital admission* – please give the date the patient was admitted to hospital during this hospital stay
- *Other relevant pathogens associated with this illness* – please state if any other organisms thought to be clinically significant to this episode have been isolated from this/these site/s.

Section iii)

Clinical presentation

- *Toxic shock syndrome* – please indicate for confirmed or possible cases, defined as 'Isolation of a group A streptococcus with hypotension (BP≤90mm Hg) and two or more of the following: renal impairment, coagulopathy, liver abnormalities, acute respiratory distress syndrome, extensive tissue necrosis, erythematous rash'.

Degree of severity - please indicate those that apply

- *Hypotensive shock* – blood pressure ≤90mm Hg
- *Renal impairment* – two-fold elevation of age-adjusted creatinine level (or higher)
- *DIC (disseminated intravascular coagulation)* - thrombocytes $<10^9$/litre
- *Liver abnormality* – raised sGOT, sGPT or two-fold elevation of bilirubin levels (or higher)
- *Soft tissue necrosis* - fasciitis, myositis or gangrene

Clinical management

- *Admitted to ITU* – please indicate if the patient has as a result of this infection been admitted to an intensive care/therapy unit and how many days were spent in ITU within the first week following GAS isolation
- *Surgical intervention* – please describe, using OPCS-4 codes for surgical procedure/s if available

Outcome – at one week after initial GAS isolation

Section iv)

Risk factors - please tick any that apply, noting any other possible risk factors, including disease or treatment-related immunosuppression and previous GAS disease (within the 14 days prior to this isolation) in the 'other risk factors' box. Please describe any surgical procedure/s, giving OPCS-4 codes if available.

Other epidemiological information

- *Occupation* – please state type if occupational exposure is thought to have possibly occurred
- *Admitted from an institution* – please indicate if the patient was admitted to hospital directly from another closed institution and state what type and which country if outside UK (e.g. transfer from Greek hospital, nursing home, prison)
- *Hospital acquired infection* – defined as infection occurring ≥48 hours after hospital admission (including time in originating hospital in the case of transfer)

If you have any queries on this questionnaire or surveillance programme, please contact Dr Androulla Efstratiou 020 8200 4400 ext.4270/4288.

Thank you for your assistance in this surveillance programme

European Commission DG RTD QLK2.CT.2002.01398

10.2. European Standard Population

Age group (years)	European Standard Population
0	1,600
1-4	6,400
5-9	7,000
10-14	7,000
15-19	7,000
20-24	7,000
25-29	7,000
30-34	7,000
35-39	7,000
40-44	7,000
45-49	7,000
50-54	7,000
55-59	6,000
60-64	5,000
65-69	4,000
70-74	3,000
75-79	2,000
80-84	1,000
85+	1,000
Total	**100,000**